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(54) Title: **T CELL EPITOPES OF THE MAJOR ALLERGENS FROM AMBROSIA ARTEMISIIFOLIA**

## (57) Abstract

The present invention provides isolated peptides of the major protein allergens of *Ambrosia artemisiifolia*, or short ragweed pollen. Peptides within the scope of the invention comprise at least one T cell epitope, or preferably at least two T cell epitopes of a protein allergen selected from the allergens Amb a I.1, Amb a I.2, Amb a I.3, Amb a I.4 and Amb a II. Modified peptides having similar or enhanced therapeutic properties as the corresponding, naturally-occurring allergen or portion thereof, but having reduced side effects are disclosed. The invention also provides nucleic acids having sequences encoding peptides of the invention. Methods of treatment or of diagnosis of sensitivity to ragweed pollen allergens in an individual and therapeutic compositions comprising one or more peptides of the invention are also provided including multipeptide formulations for human therapeutic use.

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## T CELL EPITOPEs OF THE MAJOR ALLERGENs FROM AMBROSIA ARTEMISIIFOLIA

### Background of the Invention

5        *Ambrosia artemisiifolia* or short ragweed pollen is the major cause of late summer hayfever in North America and Canada and considerable effort has been expended in trying to identify the major allergens produced by this species. *Amb a I* or Antigen E (AgE) has been reported to be the predominant allergen. (King, T.P., et al. *Biochemistry*, 3:458 (1964)). AgE has been characterized and reported to be a nonglycosylated protein of 38kD molecular mass (King, T.P., *Adv. Immunol.* 23:77 (1976); King, T.P., et al., *Arch. Biochem. Biophys.*, 212:127 (1981)). An immunochemically related protein, *Amb a II* (AgK), has been reported to have similar properties (King, T.P., *Adv. Immunol.* 23:77 (1976); King, T.P., *Biochemistry*, 11:367 (1972)). *Amb a I* or AgE can be purified using conventional chromatographic or biochemical techniques. However, it has been reported that due to 10 cleavage of the 38 kD single-chain precursors by the action of a trypsin-like pollen protease, purification often results in the isolation of two noncovalently associated chains of 26 and 12 kD molecular mass, designated  $\alpha$  and  $\beta$ , respectively (King, T.P., et al., *Arch. Biochem. Biophys.*, 212:127 (1981); King, T.P., et al., *Immunochemistry*, 11:83 (1974)). It has been reported that biochemically purified *Amb a I* has been used as an 15 immunogen to produce murine monoclonal antibodies (mAb) as well as rabbit polyclonal antisera reactive with both the native (Olson, J.R., and D.G. Klapper, *J. Immunol.*, 136:2109 (1986)) and denatured (Smith, et al., *Molec. Immunol.*, 25:355 (1988)) protein.

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Early studies using skin tests with *Amb a I*-depleted pollen extract led to the estimation that at least 90% of the allergenic activity in ragweed pollen can be attributed to *Amb a I* (King, T.P., et al., *Biochemistry*, 3:458 (1964); King, T.P., et al., *Immunol.*, 23:77 (1976)). Competition experiments using murine mAb to inhibit the binding of *Amb a I* by human IgE in ELISA assays have confirmed that *Amb a I* binds a substantial proportion of human ragweed allergic IgE (Olson, J.R., et al., *J. Immunol.*, 136:2109 (1986)). Recently, three cDNAs encoding proteins with properties of *Amb a I* were cloned and *Amb a I* was reported to be a family of homologous, but distinguishable sequences (Rafnar, T., et al., *J. Biol. Chem.*, 266:1229 (1991)). Rafnar and co-workers reported that the individual cloned members of the *Amb a I* family, designated *Amb a I.1*, *Amb a I.2*, and *Amb a I.3*, share amino acid sequence homology exceeding 80%. The fourth family member designated *Amb a I.D* (*Amb a I.4*) is disclosed in U.S.S.N. 07/529,951, filed May 29, 1990. The deduced amino acid sequence of *Amb a II* has been disclosed and reported to share approximately 65% sequence identity with the *Amb a I* multigene family of allergens. (Rogers, B. L., et al., *Journal of Immunology* 147:2547-2552 (1992)).

### Summary of the Invention

The present invention provides isolated peptides of the major protein allergens of *Ambrosia artemisiifolia* including peptides derived from the family of related proteins, previously designated *Amb a* IA, *Amb a* IB, *Amb a* IC, and *Amb a* ID. These allergens have 5 been renamed according to the IUIS approved nomenclature as *Amb a* I.1 (*Amb a* IA), *Amb a* I.2 (*Amb a* IB), *Amb a* I.3 (*Amb a* IC) and *Amb a* I.4 (*Amb a* ID). Peptides within the scope of the invention comprise at least one T cell epitope, preferably at least two T cell epitopes, 10 of a protein allergen selected from the family of *Amb a* I allergens and *Amb a* II. The invention further provides peptides comprising at least two regions, each region comprising at least one T cell epitope of a ragweed pollen allergen. The regions are derived from the 15 same or from different ragweed pollen allergens.

The invention also provides modified peptides having similar or enhanced therapeutic properties as the corresponding, naturally-occurring allergen or portion thereof, but having reduced side effects as well as modified peptides having improved properties such as 20 increased solubility and stability. Peptides of the invention are capable of modifying, in a ragweed pollen-sensitive individual to whom they are administered, the allergic response of the individual to a ragweed pollen allergen. Methods of treatment or of diagnosis of sensitivity to a ragweed pollen allergen in an individual and therapeutic compositions comprising one or more peptides of the invention are also provided and human clinical testing is described.

### Brief Description of the Drawings

Fig. 1 shows Western blot analysis of IgE binding to recombinant *Amb a* I proteins.

Fig. 2 is a graphic representation of a direct binding assay of IgE from a single 25 ragweed allergic patient to recombinant *Amb a* I and *Amb a* II proteins.

Fig. 3 is a graphic representation of the results of a direct binding assay of IgE from pooled human sera to native *Amb a* I, *Amb a* II, recombinant *Amb a* I.1, recombinant *Amb a* II and pollen extract.

Fig. 4A and 4B are graphic representations depicting the responses of lymph node 30 cells isolated from mice tolerized *in vivo* with either *Amb a* I.1 or PBS and CFA and challenged *in vitro* with various antigens.

Fig. 5A-5F are graphic representations depicting the responses of lymph node cells isolated from mice tolerized *in vivo* with *Amb a* I.1 or pollen extract, challenged with *Amb a* I.1, and tested with various antigens.

Fig. 6A-6F are graphic representations depicting the responses of lymph node cells 35 isolated from mice tolerized with pollen extract challenged with pollen extract, and tested with various antigens.

Fig. 7 shows various peptides of desired length derived from the *Amb a* I.1, *Amb a* I.2 and *Amb a* I.3 protein allergens.

Fig. 8 is a graphic representation depicting the responses of T cell lines from 39 patients primed *in vitro* to recombinant *Amb a I.1* protein and analyzed for response to various overlapping *Amb a I.1* peptides and selected *Amb a I.2* and *Amb a I.3* peptides by percent of positive responses within the individuals tested, the mean stimulation index of 5 positive responses for the peptide and the ranked sum of peptide responses.

Fig. 9 shows selected peptides of desired lengths derived from the *Amb a I.1* protein allergen.

Fig. 10 is a graphic representation depicting the responses of T cell lines from 48 patients primed *in vitro* to recombinant *Amb a I.1* protein and analyzed for response to 10 selected peptides derived from Region 1 of the *Amb a I.1* protein, by percent of positive responses within the individuals tested, the mean stimulation index of positive responses for the peptide and the ranked sum of peptide responses.

Fig. 11 is a graphic representation depicting the responses of T cell lines from 48 patients primed *in vitro* to recombinant *Amb a I.1* protein and analyzed for response to 15 selected peptides derived from Region 2 of the *Amb a I.1* protein, by percent of positive responses within the individuals tested, the mean stimulation index of positive responses for the peptide and the ranked sum of peptide responses.

Fig. 12 is a graphic representation depicting the responses of T cell lines from 48 patients primed *in vitro* to recombinant *Amb a I.1* protein and analyzed for response to 20 selected peptides derived from Region 3 of the *Amb a I.1* protein, by percent of positive responses within the individuals tested, the mean stimulation index of positive responses for the peptide and the ranked sum of peptide responses.

Fig. 13 is a graphic representation depicting the responses of T cell lines from 48 patients primed *in vitro* to recombinant *Amb a I.1* protein and analyzed for response to 25 selected peptides derived from Region 4 of the *Amb a I.1* protein, by percent of positive responses within the individuals tested, the mean stimulation index of positive responses for the peptide and the ranked sum of peptide responses.

Fig. 14 shows selected peptides of desired lengths derived from the *Amb a I.1* protein allergen and the *Amb a I.3* protein allergen.

Fig. 15 is a graphic representation depicting the responses of T cell lines from 23 patients primed *in vitro* to recombinant *Amb a I.1* protein and analyzed for response to selected peptides derived from Region 1 of the *Amb a I.1* protein, by percent of positive responses within the individuals tested, the mean stimulation index of positive responses for the peptide and the ranked sum of peptide responses.

Fig. 16 is a graphic representation depicting the responses of T cell lines from 23 patients primed *in vitro* to recombinant *Amb a I.1* protein and analyzed for response to selected peptides derived from Region 2 of the *Amb a I.1* protein, by percent of positive responses within the individuals tested, the mean stimulation index of positive responses for the peptide and the ranked sum of peptide responses.

Fig. 17 is a graphic representation depicting the responses of T cell lines from 23 patients primed *in vitro* to recombinant *Amb a 1.1* protein and analyzed for response to selected peptides derived from Region 3 of the *Amb a 1.1* protein, by percent of positive responses within the individuals tested, the mean stimulation index of positive responses for the peptide and the ranked sum of peptide responses.

Fig. 18 is a graphic representation depicting the responses of T cell lines from 23 patients primed *in vitro* to recombinant *Amb a 1.1* protein and analyzed for response to selected peptides derived from Region 4 of the *Amb a 1.1* protein, by percent of positive responses within the individuals tested, the mean stimulation index of positive responses for the peptide and the ranked sum of peptide responses.

Fig. 19 is a graphic representation depicting the responses of T cell lines of 9 patients primed *in vitro* to recombinant *Amb a 1.1* protein or recombinant *Amb a 1.3* protein and analyzed for response to selected peptides derived from *Amb a 1.1*, by percent of positive responses within the individuals tested and the mean stimulation index of positive responses for the peptide.

Fig. 20 is a graphic representation depicting the responses of T cell lines of 9 patients primed *in vitro* to recombinant *Amb a 1.1* protein or recombinant *Amb a 1.3* protein and analyzed for response to selected peptides derived from *Amb a 1.3*, by percent of positive responses within the individuals tested and the mean stimulation index of positive responses for the peptide.

Fig. 21 is a graphic representation of a direct binding assay of IgE from a single ragweed allergic patient to peptides derived from *Amb a 1*.

Fig. 22 is a graphic representation depicting the responses of T cell lines of 28 patients primed *in vitro* to recombinant *Amb a 1.1* protein and analyzed for response to selected peptides derived from *Amb a 1.1* by percent of positive responses within the individuals tested, the mean stimulation index of positive responses to the peptide and the ranked sum of peptide responses.

Fig. 23 is a graphic representation depicting the responses of T cell lines of 28 patients primed *in vitro* to recombinant *Amb a 1.1* protein and analyzed for response to selected peptides derived from Region 4 of *Amb a 1.1* by percent of positive responses within the individuals tested, the mean stimulation index of positive responses to the peptide and the ranked sum of peptide responses.

Fig. 24 is a graphic representation depicting the responses of T cell lines of 32 patients primed *in vitro* to recombinant *Amb a 1.1* protein and analyzed for response to six selected peptides derived from *Amb a 1.1* by percent of positive responses within the individuals tested, the mean stimulation index of positive responses to the peptide and the ranked sum of peptide responses.

Fig. 25 shows various peptides derived from peptide RAE 70.1 which include modifications designed to increase the solubility of the peptide.

Fig. 26 is a graphic representation depicting the response of a T cell line from patient 956.2 primed *in vitro* to *Fel d* I and analyzed for response to various peptides derived from the *Amb a* I.1 protein.

5 Fig. 27 is a graphic representation depicting the response of a T cell line from patient 119 primed *in vitro* with recombinant *Amb a* I.1 and analyzed for response to various modified peptides derived from Region 2 of the *Amb a* I.1 protein by tritiated thymidine incorporation.

10 Fig. 28 is a graphic representation depicting the response of a T cell line from patient 1199 primed *in vitro* with recombinant *Amb a* I.1 and analyzed for response to various modified peptides derived from Region 2 of the *Amb a* I.1 protein by tritiated thymidine incorporation.

15 Fig. 29 is a graphic representation depicting the response of a T cell clone generated by limiting dilution from an *Amb a* I.1 specific T cell line stimulated with the AMB 2-10.1 peptide, primed *in vitro* w/recombinant *Amb a* I.1 and analyzed for response to various modified peptides derived from Region 2 of the *Amb a* I.1 protein by tritiated thymidine incorporation.

Fig. 30 is a graphic representation depicting the percent of total histamine release in blood samples from 8 ragweed-allergic patients in response to selected peptides derived from the *Amb a* I.1 protein.

20 Fig. 31 is a graphic representation depicting the responses of T cell lines from 39 patients primed *in vitro* to purified native *Amb a* I.1 protein and analyzed for response to various overlapping *Amb a* I.1 peptides by percent of positive responses within the individuals tested (above the bar), the mean stimulation index of positive responses for the peptide (in parenthesis above the bar) and the ranked sum of peptide responses (X axis).

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#### Detailed Description of the Invention

The present invention provides isolated peptides derived from the major protein allergens of *Ambrosia artemisiifolia*. As used herein, a peptide refers to an amino acid sequence having fewer amino acids than the entire amino acid sequence of a protein from 30 which the peptide is derived. Peptides of the invention include peptides derived from *Amb a* I.1, *Amb a* I.2, *Amb a* I.3, *Amb a* I.4 and *Amb a* II which comprise at least one T cell epitope of the allergen.

Peptides comprising at least two regions, each region comprising at least one T cell epitope of a protein allergen of *Ambrosia artemisiifolia* are also within the scope of the 35 invention. Each region of such peptides is derived from the same or from different ragweed pollen allergens. Isolated peptides or regions of isolated peptides, each comprising at least two T cell epitopes of a ragweed pollen allergen are particularly desirable for increased therapeutic effectiveness. Peptides which are immunologically related (e.g., by antibody or T cell cross-reactivity) to peptides of the present invention are also within the scope of the

invention. Peptides immunologically related by antibody cross-reactivity are bound by antibodies specific for a peptide of a protein allergen of *Ambrosia artemisiifolia*. Peptides immunologically related by T cell cross-reactivity are capable of reacting with the same T cells as a peptide of the invention.

5 The present invention also pertains to a ragweed pollen allergen encoded by a nucleic acid sequence of clone IPC1/5. The full-length nucleic acid sequence of clone IPC1/5 has been determined and the encoded protein has been produced recombinantly in both the pSEM vector (as a fusion protein with  $\beta$ -galactosidase) and the pET11d vector. The recombinant protein was determined to bind approximately 10-20% of allergic serum IgE on a Western 10 blot. The protein encoded by clone IPC1/5 was found to have a high degree of amino acid sequence homology with cysteine proteinase inhibitors in man and rice. The protein has 15 66.6% homology with the rice protein oryzacystatin-I. The nucleic acid sequence and deduced amino acid sequence of the allergen encoded by clone IPC1/5 is represented in SEQ ID NO. 11 and 12.

15 Isolated proteins and isolated peptides of the invention can be produced by recombinant DNA techniques in a host cell transformed with a nucleic acid having a sequence encoding such protein or peptide. The isolated proteins and isolated peptides of the invention can also be produced by chemical synthesis. In certain limited situations, isolated peptides can be produced by chemical cleavage of a protein allergen. When a protein or 20 peptide is produced by recombinant techniques, host cells transformed with a nucleic acid having a sequence encoding the protein or peptide or the functional equivalent of the nucleic acid sequence are cultured in a medium suitable for the cells and protein or peptides can be 25 purified from cell culture medium, host cells, or both using techniques known in the art for purifying proteins and peptides including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis or immunopurification with antibodies specific for the protein or peptide, the protein allergen of *Ambrosia artemisiifolia* from which the peptide is derived, or a portion thereof. By isolated is meant that protein and peptides of the present invention are substantially free of cellular material or culture medium when 30 produced by recombinant DNA techniques, or substantially free of chemical precursors or other chemicals when synthesized chemically. Recombinant ragweed pollen proteins including recombinant *Amb a* I.1, *Amb a* I.2, *Amb a* I.3, *Amb a* I.4, and *Amb a* II have been produced.

35 Suitable expression vectors for producing recombinant protein and recombinant peptides of the invention include pTRC, pGEX, pMAL, pRIT5, pET11d and pCA. The use of pTRC, pET11d and pGEX as expression vectors will result in expression of ragweed pollen protein as an unfused protein. The use of pMAL, pRIT5, pCA and pSEM as expression vectors will result in expression of ragweed pollen protein fused to maltose E binding protein (pMAL), protein A (pRIT5), truncated protein A (pCA), or  $\beta$ -galactosidase (pSEM). Suitable expression vectors are commercially available. When produced as a

fusion protein, recombinant ragweed pollen protein can be recovered from the fusion protein through enzymatic or chemical (e.g., cyanogen bromide or dilute acid) cleavage and biochemical purification. For example, enzymatic cleavage sites for Factor Xa or thrombin can be introduced at the fusion junction between the carrier protein (e.g., Protein A) and the 5 ragweed pollen protein. Suitable host cells for expression of recombinant ragweed pollen protein include bacteria, yeast and insect or mammalian cells. Appropriate vectors for expression in yeast include YepSec, pMFa and JRY88. These vectors are also commercially available.

To obtain isolated peptides of the present invention, a ragweed pollen allergen is 10 divided into non-overlapping peptides of desired lengths or overlapping peptides of desired lengths as discussed in Example V which may be produced recombinantly, synthetically or in certain limited situations by chemical cleavage of the allergen. Peptides comprising at least one T cell epitope are capable of reducing T cell responsiveness or inducing T cell nonresponsiveness. To determine peptides comprising at least one T cell epitope, isolated 15 peptides are tested by, for example, T cell biology techniques to determine whether the peptides elicit a T cell response or induce T cell non responsiveness. Those peptides found to elicit a T cell response or induce T cell nonresponsiveness are defined as having T cell stimulating activity. As discussed in the Examples, human T cell stimulating activity can be tested by culturing T cells obtained from an individual sensitive to a ragweed pollen allergen, 20 (i.e., an individual who has an IgE mediated immune response to a ragweed pollen allergen) with a peptide derived from the allergen and determining whether proliferation of T cells occurs in response to the peptide as measured, e.g., by cellular uptake of tritiated thymidine. As described in detail in the Examples, stimulation indices for responses by T cells to peptides can be calculated as the maximum CPM in response to a peptide divided by the 25 medium control CPM. As used throughout this application, a peptide comprising at least one T cell epitope, when determined by T cell stimulation requires a stimulation index of at least 2.0. A peptide having a T cell stimulation index of 2.0 is considered useful as a therapeutic agent. Preferred peptides have a stimulation index of at least 2.5, more preferably at least 3.5, and most preferably at least 5.0.

30 In order to determine precise T cell epitopes by, for example, fine mapping techniques, a peptide having T cell stimulating activity and thus comprising at least one T cell epitope as determined by T cell biology techniques is modified by addition or deletion of amino acid residues at either the amino or carboxy terminus of the peptide and tested to determine a change in T cell reactivity to the modified peptide. If two or more peptides 35 which share an area of overlap in the native protein sequence are found to have human T cell stimulating activity, as determined by T cell biology techniques, additional peptides can be produced comprising all or a portion of such peptides and these additional peptides can be tested by a similar procedure. Following this technique, peptides are selected and produced recombinantly or synthetically. Peptides are selected based on various factors, including the

strength of the T cell response to the peptide (e.g., stimulation index), the frequency of the T cell response to the peptide in a population of individuals sensitive to ragweed pollen, and the potential cross-reactivity of the peptide with *Amb a* I family members and *Amb a* II. The physical and chemical properties of these selected peptides (e.g., solubility, stability) are examined to determine whether the peptides are suitable for use in therapeutic compositions or whether the peptides require modification as described herein. The ability of the selected peptides or selected modified peptides to stimulate human T cells (e.g., induce proliferation, lymphokine secretion) is determined.

In addition, preferred peptides of the invention do not bind immunoglobulin E (IgE) or bind IgE to a substantially lesser extent (i.e. preferably at least 100 fold and more preferably at least 1000 fold less) than the protein allergen from which the peptide is derived binds IgE. Recombinant ragweed pollen allergens including recombinant *Amb a* I.1, *Amb a* I.2, *Amb a* I.3, *Amb a* I.4, and *Amb a* II have been produced and shown to have reduced IgE binding activity as compared to the corresponding native protein allergen (See Fig. 3). The major complications of standard immunotherapy are IgE-mediated responses such as anaphylaxis. Immunoglobulin E is a mediator of anaphylactic reactions which result from the binding and cross-linking of antigen to IgE on mast cells or basophils and the release of mediators (e.g., histamine, serotonin, eosinophil chemotactic factors). Thus, anaphylaxis in a substantial percentage of a population of individuals sensitive to ragweed pollen allergen could be avoided by the use in immunotherapy of a recombinant protein, a peptide or peptides which do not bind IgE in a substantial percentage (e.g., at least about 75%) of a population of individuals sensitive to ragweed pollen allergen, or if the protein or peptide(s) binds IgE, such binding does not result in the release of mediators from mast cells or basophils. Additionally, the risk of anaphylaxis can be reduced by the use in immunotherapy of a recombinant protein, a peptide or peptides which have reduced IgE binding. Moreover, recombinant protein or peptides which have minimal IgE stimulating activity are desirable for therapeutic effectiveness. Minimal IgE stimulating activity refers to IgE production that is less than the amount of IgE production and/or IL-4 production stimulated by the native protein allergen (e.g., *Amb a* I.1).

A peptide or recombinant protein of the invention, when administered to a ragweed pollen-sensitive individual, is capable of modifying the allergic response of the individual to the allergen. Particularly, peptides of the invention comprising at least one T cell epitope of a ragweed pollen allergen or at least two regions derived from a ragweed pollen allergen each comprising at least one T cell epitope, when administered to a ragweed pollen-sensitive individual are capable of modifying the T cell response of the individual to the allergen. As used herein, modification of the allergic response of a ragweed pollen-sensitive individual to a ragweed pollen allergen can be defined as non-responsiveness or diminution in symptoms to a ragweed pollen allergen, as determined by standard clinical procedures (see e.g., Varney et al., British Medical Journal 302: 265-269 (1990)).

As a result of the work described herein, peptides derived from ragweed pollen allergens comprising at least one T cell epitope have been produced. T cell epitopes are believed to be involved in initiation and perpetuation of the immune response to ragweed pollen allergen(s) which are responsible for the clinical symptoms of ragweed pollen allergy.

5 These T cell epitopes are thought to trigger early events at the level of the T helper cell by binding to an appropriate HLA molecule on the surface of an antigen presenting cell and stimulating the relevant T cell subpopulation. These events lead to T cell proliferation, lymphokine secretion, local inflammatory reactions, the recruitment of additional immune cells to the site, and activation of the B cell cascade leading to production of antibodies. One 10 isotype of these antibodies, IgE, is fundamentally important in the development of allergic symptoms and its production is influenced early in the cascade of events, at the level of the T helper cell, by the nature of the lymphokines secreted. A T cell epitope is the basic element or smallest unit of recognition by a T cell receptor, where the epitope comprises amino acid residues essential to receptor recognition which may be contiguous and/or non-contiguous in 15 the amino acid sequence of the protein. Amino acid sequences which mimic those of T cell epitopes and which modify the allergic response to protein allergens of *Ambrosia artemisiifolia* are within the scope of this invention.

Exposure of ragweed pollen allergic patients to peptides of the present invention, in a non-immunogenic form, may induce T cell non-responsiveness of appropriate T cell 20 subpopulations such that they become non-responsive to ragweed pollen allergen(s) and do not participate in mounting an immune response upon such exposure.

While not intending to be limited to any theory, it is believed that T cell non-responsiveness (which includes reduced T cell responsiveness) is induced as a result of not providing an appropriate costimulatory signal sometimes referred to as a "second signal" 25. Briefly, it is believed that stimulation of T cells requires two types of signals, the first is the recognition by the T cell via the T cell receptor of appropriate MHC-associated processed antigens on antigen presenting cells (APCs) and the second type of signal is referred to as a costimulatory signal(s) or "second signal" which may be provided by certain competent APCs. When a composition of the invention is administered without 30 adjuvant, it is believed that competent APCs which are capable of producing the second signal or costimulatory signal are not engaged in the stimulation of appropriate T cells therefore resulting in T cell nonresponsiveness or reduced T cell responsiveness. In addition, there are a number of antibodies or other reagents capable of blocking the delivery of costimulatory signals such as the "second signal" which include, but are not 35 limited to B7 (including B7-1, B7-2, and BB-1), CD28, CTLA4, CD40 CD40L CD54 and CD11a/18 (Jenkins and Johnson, *Current Opinion in Immunology*, 5:361-367 (1993), and Clark and Ledbetter, *Nature*, 367:425-428 (1994)) Thus, a peptide of the invention may be administered in nonimmunogenic form as discussed above, in conjunction with a reagent

capable of blocking costimulatory signals such that the level of T cell nonresponsiveness is enhanced.

In addition, administration of a peptide of the present invention may modify the lymphokine secretion profile as compared with exposure to the naturally-occurring ragweed pollen allergen or portion thereof (e.g., result in a decrease of IL-4 and/or an increase in IL-2). Furthermore, exposure to a peptide of the invention may influence T cell subpopulations which normally participate in the response to ragweed pollen allergen(s) such that these T cells are drawn away from the site(s) of normal exposure to the allergen (e.g., nasal mucosa, skin, and lung) towards the site(s) of therapeutic administration of the peptide. This 10 redistribution of T cell subpopulations may ameliorate or reduce the ability of an individual's immune system to stimulate the immune response at the site of normal exposure to the ragweed pollen allergen(s), resulting in a diminution in allergic symptoms.

Isolated peptides of the invention comprise at least one T cell epitope of a protein allergen of *Ambrosia artemisiifolia* (i.e., the peptide comprises at least approximately seven 15 amino acid residues of the protein allergen). For purposes of therapeutic effectiveness, therapeutic compositions of the invention preferably comprise at least two T cell epitopes of a ragweed pollen allergen. Accordingly, isolated peptides of the invention preferably comprise at least two T cell epitopes (i.e., the peptide comprises at least approximately eight amino acid residues, and preferably fifteen amino acid residues). Additionally, isolated 20 peptides of the invention preferably comprise a sufficient percentage of the T cell epitopes of the entire protein allergen such that upon administration of the peptide to an individual sensitive to ragweed pollen, T cells of the individual are rendered non-responsive to the protein allergen. Isolated peptides of the invention comprising up to approximately 45 amino acid residues in length, and most preferably up to approximately 30 amino acid residues in 25 length are particularly desirable as increases in length may result in difficulty in peptide synthesis as well as retention of an undesirable property (e.g., immunoglobulin binding or enzymatic activity) due to maintenance of conformational similarity between the peptide and the protein allergen from which it is derived. All of the peptides shown in Fig. 8 were found to have human T cell stimulating activity.

To determine whether a peptide (candidate peptide) or a combination of candidate 30 peptides are likely contain a sufficient percentage of T cell epitopes of ragweed protein antigen to induce non-responsiveness in a substantial percentage of a population of individuals sensitive to the protein antigen, an algorithm can be used. In accordance with one such algorithm, a human T cell stimulation index (discussed above) for the peptide(s) in an *in vitro* T cell proliferation assay is calculated for each individual tested in a population of 35 individuals sensitive to ragweed protein allergen. The remaining peptides in the *in vitro* T cell proliferation assay are overlapping peptides (overlapping by between about 5 - 10 amino acid residues) which cover the remainder of the protein not covered by the candidate peptide(s), which peptides are at least 12 amino acids long and which are preferably no longer than 30

and more preferably no longer than 25 amino acid residues in length. A human T cell stimulation index for each such remaining peptide in the set of peptides produced in the *in vitro* T-cell proliferation assay with T-cells obtained from each individual in the population of individuals tested is calculated and added together. For each individual, the human T cell 5 stimulation index for the candidate peptide(s) is divided by the sum of the human T cell stimulation indices of the remaining peptides in the set of peptides tested to determine a percent. This percent is obtained for at least twenty (20) and preferably at least thirty (30) individuals sensitive to the protein antigen of interest and a mean percent is determined (the 10 percentage of positive T cell responses (S.I. greater than or equal to 2.0) in response to the candidate peptide or combination of candidate peptides). A mean percent of about 10% or greater for the candidate peptide(s) together with a percent positive of at least about 60%, preferably about 75% and more preferably about 90%, or most preferably 100%, indicates 15 that the candidate peptide(s) selected is likely to contain a sufficient percentage of T cell epitopes to induce T cell non responsiveness in a substantial percentage of a population of individuals sensitive to TRFP.

Preferred peptides comprise all or a portion of the areas of major T cell reactivity within the *Amb a* I.1 protein allergen, i.e., Region 1, Region 2, Region 3 and Region 4. Each area is broadly defined as follows: Region 1 comprises amino acid residues 48-107; Region 2 comprises amino acid residues 171-216; Region 3 comprises amino acid residues 278-322; 20 and Region 4 comprises amino acid residues 331-377. Preferred areas of major T cell reactivity within each Region comprise: amino acid residues 57-101; amino acid residues 182-216; amino acid residues 280-322; and amino acid residues 342-377. Similar areas of major T cell reactivity can be found within the other *Amb a* I family members (i.e., *Amb a* I.2, *Amb a* I.3 and *Amb a* I.4), and *Amb a* II. As shown in Example VIII, the *Amb a* I protein 25 allergens and *Amb a* II demonstrate a high degree of T cell cross-reactivity. Given this cross-reactivity, shared areas of major T cell reactivity and shared T cell epitopes are likely to be found in conserved regions between *Amb a* I and the remaining *Amb a* I family members and *Amb a* II. For example, *Amb a* I.1 stimulated T cells have been shown to recognize both *Amb a* I.1 derived peptides and homologous *Amb a* I.3 derived peptides (See Example IX). 30 Similarly, *Amb a* I.3 stimulated T cell recognize both *Amb a* I.1 and *Amb a* I.3 derived peptides.

Preferred ragweed pollen peptides comprise all or a portion of the following peptides: RAE 67.1 (SEQ ID NO:13); RAE 57.1 (SEQ ID NO:14); RAE 24.E (SEQ ID NO:15); RAE 24.1 (SEQ ID NO:16); RAE 22.E (SEQ ID NO:17); RAE 22.E-1 (SEQ ID NO:18); RAE 3.D 35 (SEQ ID NO:19); RAE 3.1 (SEQ ID NO:20); RAE 22.E-2 (SEQ ID NO:21); RAE 5.D (SEQ ID NO:22); RAE 6.D (SEQ ID NO:23); RAE 6.1 (SEQ ID NO:24); RAE 7.D (SEQ ID NO:25); RAE 7.D-1 (SEQ ID NO:26); RAE 40.1-6 (SEQ ID NO:27); RAE 40.1-5 (SEQ ID NO:28); RAE 40.1-4 (SEQ ID NO:29); RAE 40.D (SEQ ID NO:30); RAE 40.1 (SEQ ID NO:31); RAE 61.1 (SEQ ID NO:32); RAE 80.1 (SEQ ID NO:33); RAE 45.1 (SEQ ID

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NO:34); RAE 75.1 (SEQ ID NO:35); RAE 62.1 (SEQ ID NO:36); RAE 69.1 (SEQ ID NO:37); RAE 69.1-1 (SEQ ID NO:38); RAE 69.1-2 (SEQ ID NO:39); RAE 69.1-3 (SEQ ID NO:40); RAE 70.1-3 (SEQ ID NO:41); RAE 70.1-2 (SEQ ID NO:42); RAE 70.1-1 (SEQ ID NO:43); RAE 70.1 (SEQ ID NO:44); RAE 71.1 (SEQ ID NO:45); RAE 65.1 (SEQ ID NO:46); RAE 63.1 (SEQ ID NO:47); RAE 76.1 (SEQ ID NO:48); RAE 27.1 (SEQ ID NO:49); RAE 66.1 (SEQ ID NO:50); RAE 66.1-1 (SEQ ID NO:51); RAE 66.1-2 (SEQ ID NO:52); RAE 66.1-3 (SEQ ID NO:53); RAE 64.1-3 (SEQ ID NO:54); RAE 64.1-2 (SEQ ID NO:55); RAE 64.1-1 (SEQ ID NO:56); RAE 64.1 (SEQ ID NO:57); RAE 73.1 (SEQ ID NO:58); RAE 74.1 (SEQ ID NO:59); RAE 74.1-1 (SEQ ID NO:60); RAE 29.1 (SEQ ID NO:61); RAE 29.1-1 (SEQ ID NO:62); RAE 28+29 (SEQ ID NO:63); RAE 29.1-2 (SEQ ID NO:64); RAE 29.1-3 (SEQ ID NO:65); RAE 29.1-4 (SEQ ID NO:66); RAE 28.1-3 (SEQ ID NO:67); RAE 28.1-2 (SEQ ID NO:68); RAE 28.1-1 (SEQ ID NO:69); RAE 28.1 (SEQ ID NO:70); RAE 20.1 (SEQ ID NO:71); RAE 20.1-3 (SEQ ID NO:72); RAE 20.1-2 (SEQ ID NO:73); RAE 20.1-1 (SEQ ID NO:74); RAE 21.1 (SEQ ID NO:75); RAE 17.1 (SEQ ID NO:76); RAE 55.1 (SEQ ID NO:77); RAE 76.6 (SEQ ID NO:78); RAE 67.15 (SEQ ID NO:79); RAE 45.15 (SEQ ID NO:80); RAE 27.15 (SEQ ID NO:81); AMB 1-1.1 (SEQ ID NO:85); AMB 1-2.1 (SEQ ID NO:86); AMB 1-3.1 (SEQ ID NO:87); AMB 1-4.1 (SEQ ID NO:84); AMB 1-5.1 (SEQ ID NO:83); AMB 1-6.1 (SEQ ID NO:82); AMB 1-4.15 (SEQ ID NO:88); AMB 1-2.15 (SEQ ID NO:89); AMB 2-4.1 (SEQ ID NO:90); AMB 2-3.1 (SEQ ID NO:91); AMB 2-5.1 (SEQ ID NO:92); AMB 2-6.1 (SEQ ID NO:93); AMB 2-2.1 (SEQ ID NO:94); AMB 2-1.1 (SEQ ID NO:95); AMB 2-7.1 (SEQ ID NO:96); AMB 2-8.1 (SEQ ID NO:97); AMB 2-9.1 (SEQ ID NO:98); AMB 2-10.1 (SEQ ID NO:99); AMB 2-11.1 (SEQ ID NO:100); AMB 2-1.15 (SEQ ID NO:101); AMB 3-4.1 (SEQ ID NO:103); AMB 3-5.1 (SEQ ID NO:102); AMB 3-3.1 (SEQ ID NO:104); AMB 3-2.1 (SEQ ID NO:105); AMB 3-1.1 (SEQ ID NO:106); AMB 3-4.15 (SEQ ID NO:107); AMB 3-1.15 (SEQ ID NO:108); AMB 4-8.1 (SEQ ID NO:109); AMB 4-9.1 (SEQ ID NO:110); AMB 4-6.1 (SEQ ID NO:111); AMB 4-5.1 (SEQ ID NO:112); AMB 4-3.1 (SEQ ID NO:113); AMB 4-2.1 (SEQ ID NO:114); AMB 4-1.1 (SEQ ID NO:115); AMB 4-3.15 (SEQ ID NO:116); Amb 2-18.1 (SEQ ID NO:126); Amb 2-19.1 (SEQ ID NO:127); Amb 2-20.1 (SEQ ID NO:128); Amb 2-21.1 (SEQ ID NO:129); Amb 2-22.1 (SEQ ID NO:130); Amb 2-23.1 (SEQ ID NO:131); Amb 2-26.1 (SEQ ID NO:132); Amb 28.1 (SEQ ID NO:133); Amb 2-30.1 (SEQ ID NO:134); Amb 2-32.1 (SEQ ID NO:135); Amb 2-33.1 (SEQ ID NO:136); Amb 2-34.1 (SEQ ID NO:137); Amb 2-35.1 (SEQ ID NO:138); Amb 2-36.1 (SEQ ID NO:139); Amb 2-37.1 (SEQ ID NO:140); Amb 2-38.1 (SEQ ID NO:141); AMB 4-9.1EP (SEQ ID NO:142); AMB 4-9.1NP (SEQ ID NO:143); AMB 4-9.1AP (SEQ ID NO:144); AMB 4-9.1SP (SEQ ID NO:145); AMB 4-9.1QP (SEQ ID NO:146); AMB 4-9.1DA (SEQ ID NO:147); amb 4-9.1DS (SEQ ID NO:148); AMB 4-9.1DG (SEQ ID NO:149); and RA-02.1 (SEQ ID NO:150), the amino acid sequences of such peptides being shown in Figs. 7, 14, 23, 24 and 25. Particularly preferred peptides comprise all or a portion of the following peptides: AMB 1-2.1 (SEQ ID NO:86);

AMB 2-6.1 (SEQ ID NO:93); AMB 2-4.1 (SEQ ID NO:90); Amb 2-36.1 (SEQ ID NO:139); Amb 2-38.1 (SEQ ID NO:141); RA-02.1 (SEQ ID NO:150); AMB 2-9.1 (SEQ ID NO:98); AMB 3-5.1 (SEQ ID NO:102); and AMB 4-9.1 (SEQ ID NO:110).

Another embodiment of the present invention provides peptides comprising at least two regions, each region comprising at least one T cell epitope of a protein allergen of *Ambrosia artemisiifolia* (e.g., each region comprises at least approximately seven amino acid residues). These peptides comprising at least two regions can comprise as many amino acid residues as desired and preferably comprise at least about 7, more preferably at least about 15, even more preferably about 30 and most preferably at least about 40 amino acid residues of a ragweed pollen allergen. Each region of such peptide preferably comprises up to 45 amino acid residues in length, more preferably up to 40 residues in length and most preferably up to 30 amino acid residues in length as increases in length of a region may result in difficulty in peptide synthesis as well as retention of an undesirable property (e.g., immunoglobulin binding or enzymatic activity) due to maintenance of conformational similarity between the peptide and the protein allergen from which it is derived. If desired, the amino acid sequences of the regions can be produced and joined by a linker to increase sensitivity to processing by antigen-presenting cells. Such linker can be any non-epitope amino acid sequence or other appropriate linking or joining agent. To obtain preferred peptides comprising at least two regions, each comprising at least one T cell epitope, the regions are arranged in a configuration different from a naturally-occurring configuration of the regions in the allergen. For example, the regions containing T cell epitope(s) can be arranged in a noncontiguous configuration and can preferably be derived from the same protein allergen. Noncontiguous is defined as an arrangement of regions containing T cell epitope(s) which is different than that of an amino acid sequence present in the protein allergen from which the regions are derived. Furthermore, the noncontiguous regions containing T cell epitopes can be arranged in a nonsequential order (e.g., in an order different from the order of the amino acids of the native protein allergen from which the region containing T cell epitope(s) are derived in which amino acids are arranged from an amino terminus to a carboxy terminus). A peptide can comprise at least 15%, at least 30%, at least 50% or up to 100% of the T cell epitopes of a ragweed pollen allergen.

The individual peptide regions can be produced and tested to determine which regions bind immunoglobulin E specific for a ragweed pollen allergen and which of such regions would cause the release of mediators (e.g., histamine) from mast cells or basophils. Those peptide regions found to bind immunoglobulin E and cause the release of mediators from mast cells or basophils in greater than approximately 10-15% of the allergic sera tested are preferably not included in the peptide regions arranged to form peptides of the invention.

Preferred peptides of the invention comprise two or more regions derived from the same or from different ragweed pollen allergens (e.g., *Amb a I.1*, *Amb a I.2*, *Amb a I.3*, *Amb a I.4* and *Amb a II*). For example, one region can be derived from *Amb a I.1* and one region

can be derived from *Amb a* 1.2; one region can be derived from *Amb a* 1.1 and one region can be derived from *Amb a* 1.3; one region can be derived from *Amb a* 1.1 and one region can be derived from *Amb a* 1.4; one region can be derived from *Amb a* 1.2 and one region can be derived from *Amb a* 1.3; one region can be derived from *Amb a* 1.2 and one region can be derived from *Amb a* 1.4; one region can be derived from *Amb a* 1.3 and one region can be derived from *Amb a* 1.4; one region can be derived from *Amb a* 1.1 and one region can be derived from *Amb a* II; one region can be derived from *Amb a* 1.2 and one region can be derived from *Amb a* II; one region can be derived from *Amb a* 1.3 and one region can be derived from *Amb a* II; and one region can be derived from *Amb a* 1.4 and one region can be derived from *Amb a* II. Alternatively, the regions can be derived from the same protein allergen, e.g., *Amb a* 1.1 and *Amb a* 1.1, etc.

Regions of a peptide of the invention preferably comprise all or a portion of Region 1, Region 2, Region 3 and Region 4 of *Amb a* 1.1, and the above discussed preferred areas of major T cell reactivity within each Region. If Region 1, 2, 3 or 4 is found to bind IgE and cause the release of mediators from mast cells or basophils, then it is preferred that more than one region of the peptide comprise such Region and that the various regions of the peptide do not bind IgE or cause release of mediators from mast cells or basophils. Examples of preferred regions include: AMB 1-1.1 (SEQ ID NO:85); AMB 1-2.1 (SEQ ID NO:86); AMB 1-3.1 (SEQ ID NO:87); AMB 1-4.1 (SEQ ID NO:84); AMB 1-5.1 (SEQ ID NO:83); AMB 1-6.1 (SEQ ID NO:82); AMB 1-4.15 (SEQ ID NO:88); AMB 1-2.15 (SEQ ID NO:89); AMB 2-2.4.1 (SEQ ID NO:90); AMB 2-3.1 (SEQ ID NO:91); AMB 2-5.1 (SEQ ID NO:92); AMB 2-6.1 (SEQ ID NO:93); AMB 2-2.1 (SEQ ID NO:94); AMB 2-1.1 (SEQ ID NO:95); AMB 2-7.1 (SEQ ID NO:96); AMB 2-8.1 (SEQ ID NO:97); AMB 2-9.1 (SEQ ID NO:98); AMB 2-10.1 (SEQ ID NO:99); AMB 2-11.1 (SEQ ID NO:100); AMB 2-1.15 (SEQ ID NO:101); AMB 3-4.1 (SEQ ID NO:103); AMB 3-5.1 (SEQ ID NO:102); AMB 3-3.1 (SEQ ID NO:104); AMB 3-2.1 (SEQ ID NO:105); AMB 3-1.1 (SEQ ID NO:106); AMB 3-4.15 (SEQ ID NO:107); AMB 3-1.15 (SEQ ID NO:108); AMB 4-8.1 (SEQ ID NO:109); AMB 4-9.1 (SEQ ID NO:110); AMB 4-6.1 (SEQ ID NO:111); AMB 4-5.1 (SEQ ID NO:112); AMB 4-3.1 (SEQ ID NO:113); AMB 4-2.1 (SEQ ID NO:114); AMB 4-1.1 (SEQ ID NO:115); AMB 4-3.15 (SEQ ID NO:116); RA-02.1 (SEQ ID NO:150); Amb 2-36.1 (SEQ ID NO:139); and Amb 2-38.1 (SEQ ID NO:141), the amino acid sequences of such regions being shown in Fig. 14, Fig. 24 or Fig. 25, or portions of said regions comprising at least one T cell epitope.

Preferred peptides comprise various combinations of two or more regions, each region comprising all or a portion of Region 1, Region 2, Region 3 or Region 4 of *Amb a* 1.1. Preferred peptides comprise various combinations of two or more regions, each region having an amino acid sequence as shown in Fig. 14, such combination of regions including the following: AMB 4-6.1 and RAE 70.1 (SEQ ID NO:111 and SEQ ID NO:44); AMB 4-6.1 and AMB 2-5.1 (SEQ ID NO:111 and SEQ ID NO:92); AMB 4-9.1 and AMB 2-5.1 (SEQ ID NO:110 and SEQ ID NO:92); AMB 4-9.1 and RAE 70.1 (SEQ ID NO:110 and SEQ ID NO:44).

NO:44); AMB 4-6.1, AMB 2-5.1 and AMB 1-2.1 (SEQ ID NO:111, SEQ ID NO:92 and SEQ ID NO:86); AMB 4-9.1, AMB 2-5.1 and AMB 1-2.1 (SEQ ID NO:110, SEQ ID NO:92 and SEQ ID NO:86); AMB 4-6.1, RAE 70.1 and AMB 1-2.1 (SEQ ID NO:111, SEQ ID NO:44 and SEQ ID NO:86); AMB 4-9.1, RAE 70.1 and AMB 1-2.1 (SEQ ID NO:110, SEQ ID NO:44 and SEQ ID NO:86); AMB 4-6.1, RAE 70.1, AMB 1-2.1 and AMB 3-5.1 (SEQ ID NO:111, SEQ ID NO:44, SEQ ID NO:86 and SEQ ID NO:102); AMB 4-9.1, RAE 70.1, AMB 1-2.1 and AMB 3-5.1 (SEQ ID NO:110, SEQ ID NO:44, SEQ ID NO:86 and SEQ ID NO:102); AMB 4-6.1, AMB 2-5.1, AMB 1-2.1 and AMB 3-5.1 (SEQ ID NO:111, SEQ ID NO:92, SEQ ID NO:86 and SEQ ID NO:102); AMB 4-9.1, AMB 2-5.1, AMB 1-2.1 and AMB 3-5.1 (SEQ ID NO:110, SEQ ID NO:92, SEQ ID NO:86 and SEQ ID NO:102); AMB 4-6.1, RAE 70.1, AMB 1-2.1 and AMB 3-4.1 (SEQ ID NO:111, SEQ ID NO:44, SEQ ID NO:86 and SEQ ID NO:103); AMB 4-9.1, RAE 70.1, AMB 1-2.1 and AMB 3-4.1 (SEQ ID NO:110, SEQ ID NO:44, SEQ ID NO:86 and SEQ ID NO:103); AMB 4-6.1, AMB 2-5.1, AMB 1-2.1 and AMB 3-4.1 (SEQ ID NO:111, SEQ ID NO:92, SEQ ID NO:86 and SEQ ID NO:103); AMB 4-9.1, AMB 2-5.1, AMB 1-2.1 and AMB 3-4.1 (SEQ ID NO:110, SEQ ID NO:92, SEQ ID NO:86 and SEQ ID NO:103); AMB 2-1.15 and AMB 4-3.15 (SEQ ID NO:101, and SEQ ID NO:116); AMB 1-2.15, AMB 2-1.15 and AMB 4-3.15 (SEQ ID NO:89, SEQ ID NO:101, and SEQ ID NO:116); and AMB 1-2.15, AMB 2-1.15, AMB 4-3.15 and AMB 3-4.15 (SEQ ID NO:89, SEQ ID NO:101, SEQ ID NO:116 and SEQ ID NO:107).

Peptides of protein allergens of *Ambrosia artemisiifolia* within the scope of the invention can be used in methods of treating and preventing allergic reactions to ragweed pollen allergens. Thus, one aspect of the present invention provides therapeutic compositions comprising a peptide of *Amb a* 1.1, *Amb a* 1.2, *Amb a* 1.3, *Amb a* 1.4 or *Amb a* II including at least one T cell epitope, or preferably at least two T cell epitopes, and a pharmaceutically acceptable carrier or diluent. In another aspect, the therapeutic composition comprises a pharmaceutically acceptable carrier or diluent and a peptide comprising at least two regions, each region comprising at least one T cell epitope of a ragweed pollen allergen and is derived from the same or from different ragweed pollen allergens.

Administration of the therapeutic compositions of the present invention to desensitize an individual can be carried out using known techniques. For example, a peptide derived from a ragweed pollen allergen comprising at least one T cell epitope can be administered in combination with an appropriate diluent, or carrier. Preferably, peptides are administered in soluble form. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Pharmaceutically acceptable carriers include polyethylene glycol (Wie et al., *International Archives of Allergy and Applied Immunology* 64: 84-99 (1981)) and liposomes (Strejan et al., *Journal of Neuroimmunology* 2: 27 (1984)). For purposes of inducing T-cell non-responsiveness (or reduced T cell responsiveness) in an individual, the therapeutic composition is preferably administered in non-immunogenic form, e.g., one that does not

include adjuvant. Such compositions will generally be administered by injection (e.g., intravenous, subcutaneous, intramuscular), oral administration, (e.g., as in the form of a capsule), inhalation, transdermal application or rectal administration. Preferably, therapeutic compositions are administered subcutaneously.

5 In addition, isolated and purified native ragweed pollen protein allergens (see, Example 1 for isolation and purification of ragweed pollen protein antigens) or portions thereof, can be administered orally.

10 The therapeutic compositions of the invention are administered to ragweed pollen-sensitive individuals at dosages and for lengths of time effective to reduce sensitivity (i.e., reduce the allergic response) of an individual to a ragweed pollen allergen. A therapeutically effective amount of one or more of the same or of different therapeutic compositions can be administered simultaneously or sequentially to a ragweed pollen-sensitive individual. Effective amounts of the therapeutic compositions will vary according to factors such as the 15 degree of sensitivity of the individual to ragweed pollen allergens, the age, sex, and weight of the individual, and the ability of the peptide to stimulate a T cell response in the individual.

15 For subcutaneous injection of one or more therapeutic compositions of the invention, preferably about 1 mg- 3 mg and more preferably from about 20mg-1.5 mg, and even more preferably about 50 mg- 750 mg of each active component (peptide) per dosage unit may be administered. It is especially advantageous to formulate parenteral compositions in unit 20 dosage form for ease of administration and uniformity of dosage. Unit dosage form as used herein refers to physically discrete units suited as unitary dosages for human subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the desired pharmaceutical carrier. The specification for the novel unit dosage forms of the invention are dictated by and directly 25 dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of human subjects.

30 Dosage regimen may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered over the course of days, weeks, months or years, or the dose may be proportionally increased or reduced with each subsequent injection as indicated by the exigencies of the therapeutic situation. In one preferred therapeutic regimen, subcutaneous injections of therapeutic compositions are given once a week for 3-6 weeks. The dosage may remain constant for each injection or may increase or decrease with each subsequent injection. A booster injection may be administered at 35 intervals of about three months to about one year after initial treatment and may involve only a single injection or may involve another series of injections similar to that of the initial treatment.

To administer a composition of the invention by other than parenteral administration, (i.e. oral administration) it may be necessary to coat the composition with, or co-administer

the composition with, a material to prevent its inactivation or enhance its absorption and bioavailability. For example, a peptide formulation may be co-administered with enzyme inhibitors or in liposomes. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEP) and trasylol. Liposomes include water-in-oil-in-water emulsions as well as conventional liposomes (Strejan et al., (1984) *J. Neuroimmunol.*, 7:27). When a peptide is suitably protected, the peptide may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The peptide and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the individual's diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, solutions, gels, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the composition and preparations may, of course, be varied and may conveniently be between about 5 to 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. In addition, the active compound may be incorporated into sustained-release or controlled release (steady state or pulsatile release) preparations and formulations.

In yet another aspect of the present invention, a composition is provided comprising at least two peptides (e.g., a physical mixture of at least two peptides), each comprising at least one T cell epitope of a protein allergen of *Ambrosia artemisiifolia*. Compositions comprising several peptides or combinations of separate peptides can include as many peptides as desired (e.g., 5, 6, 7...) for therapeutic efficacy. The peptides are derived from the same or from different ragweed pollen allergens. Such compositions can be administered in the form of a therapeutic composition with a pharmaceutically acceptable carrier or diluent. Preferably, peptides are administered in soluble form. A therapeutically effective amount of one or more of such compositions can be administered simultaneously or sequentially, preferably subcutaneously, to a ragweed pollen-sensitive individual to desensitize or tolerize the individual to ragweed pollen.

Preferred compositions and preferred combinations of peptides which can be administered simultaneously or sequentially (comprising peptides having amino acid sequences shown in Fig. 14) include the following combinations: AMB 4-6.1 and RAE 70.1 (SEQ ID NO:111 and SEQ ID NO:44); AMB 4-6.1 and AMB 2-5.1 (SEQ ID NO:111 and SEQ ID NO:92); AMB 4-9.1 and AMB 2-5.1 (SEQ ID NO:110 and SEQ ID NO:92); AMB 4-9.1 and RAE 70.1 (SEQ. ID NO:110 and SEQ ID NO:44); AMB 4-6.1, AMB 2-5.1 and AMB 1-2.1 (SEQ ID NO:111, SEQ ID NO:92 and SEQ ID NO:86); AMB 4-9.1, AMB 2-5.1 and AMB 1-2.1 (SEQ ID NO:110, SEQ ID NO:92 and SEQ ID NO:86); AMB 4-6.1, RAE 70.1 and AMB 1-2.1 (SEQ ID NO:111, SEQ ID NO:44 and SEQ ID NO:86); AMB 4-9.1, RAE 70.1 and AMB 1-2.1 (SEQ ID NO:110, SEQ ID NO:44 and SEQ ID NO:86); AMB 4-

6.1, RAE 70.1, AMB 1-2.1 and AMB 3-5.1 (SEQ ID NO:111, SEQ ID NO:44, SEQ ID NO:86 and SEQ ID NO:102); AMB 4-9.1, RAE 70.1, AMB 1-2.1 and AMB 3-5.1 (SEQ ID NO:110, SEQ ID NO:44, SEQ ID NO:86 and SEQ ID NO:102); AMB 4-6.1, AMB 2-5.1, AMB 1-2.1 and AMB 3-5.1 (SEQ ID NO:111, SEQ ID NO:92, SEQ ID NO:86 and SEQ ID NO:102); AMB 4-9.1, AMB 2-5.1, AMB 1-2.1 and AMB 3-5.1 (SEQ ID NO:110, SEQ ID NO:92, SEQ ID NO:86 and SEQ ID NO:102); AMB 4-9.1, AMB 2-5.1, AMB 1-2.1 and AMB 3-4.1 (SEQ ID NO:111, SEQ ID NO:44, SEQ ID NO:86 and SEQ ID NO:103); AMB 4-9.1, RAE 70.1, AMB 1-2.1 and AMB 3-4.1 (SEQ ID NO:111, SEQ ID NO:44, SEQ ID NO:86 and SEQ ID NO:103); AMB 4-6.1, AMB 2-5.1, AMB 1-2.1 and AMB 3-4.1 (SEQ ID NO:111, SEQ ID NO:92, SEQ ID NO:86 and SEQ ID NO:103); AMB 4-9.1, AMB 2-5.1, AMB 1-2.1 and AMB 3-4.1 (SEQ ID NO:110, SEQ ID NO:92, SEQ ID NO:86 and SEQ ID NO:103); AMB 1-2.1 and AMB 3-4.1 (SEQ ID NO:110, SEQ ID NO:92, SEQ ID NO:86 and SEQ ID NO:103); AMB 2-1.15 and AMB 4-3.15 (SEQ ID NO:101, and SEQ ID NO:116); AMB 1-2.15, AMB 2-1.15 and AMB 4-3.15 (SEQ ID NO:89, SEQ ID NO:101, and SEQ ID NO:116); and AMB 1-2.15, AMB 2-1.15, AMB 4-3.15 and AMB 3-4.15 (SEQ ID NO:89, SEQ ID NO:101, SEQ ID NO:116 and SEQ ID NO:107).

Particularly preferred compositions and preferred combinations of peptides for therapeutic administration include the following combinations: AMB 1-2.1 and AMB 4-9.1 (SEQ ID NO:86 and SEQ ID NO:110); AMB 1-2.1, Amb 2-38.1 and AMB 4-9.1 (SEQ ID NO:86, SEQ ID NO:141 and SEQ ID NO:110); AMB 1-2.1, Amb 2-38.1, AMB 4-9.1 and AMB 2-4.1 (SEQ ID NO:86, SEQ ID NO:141, SEQ ID NO:110 and SEQ ID NO:90); AMB 1-2.1, Amb 2-38.1, AMB 4-9.1, AMB 2-4.1 and AMB 3-5.1 (SEQ ID NO:86, SEQ ID NO:141, SEQ ID NO:110, SEQ ID NO:90, and SEQ ID NO:102); AMB 1-2.1, Amb 2-36.1 and AMB 4-9.1 (SEQ ID NO:86, SEQ ID NO:139 and SEQ ID NO:110); AMB 1-2.1, Amb 2-36.1, AMB 4-9.1 and AMB 2-4.1 (SEQ ID NO:86, SEQ ID NO:139, SEQ ID NO:110 and SEQ ID NO:90); and AMB 1-2.1, Amb 2-36.1, AMB 4-9.1, AMB 2-4.1 and AMB 3-5.1 (SEQ ID NO:86, SEQ ID NO:139, SEQ ID NO:110, SEQ ID NO:90, and SEQ ID NO:102).

Another aspect of this invention pertains to a multipeptide formulation suitable for pharmaceutical administration to ragweed sensitive individuals. The multipeptide formulation includes at least two or more peptides of ragweed pollen protein allergen having human T cell stimulating activity in an *in vitro* T cell proliferation assay (i.e., comprising at least one T cell epitope). Special considerations when preparing a multipeptide formulation include maintaining the solubility and stability of all peptides in the formulation at a physiologically acceptable pH. This requires choosing one or more pharmaceutically acceptable carriers such as excipients which are compatible with all the peptides in the multipeptide formulation. For example, suitable excipients include sterile water, sodium phosphate, mannitol or both sodium phosphate and mannitol or any combination thereof. Additionally due to the potential for dimerization of the peptides in a multipeptide formulation, there may also be included an agent such as EDTA to prevent dimerization. Alternatively, any material or procedures known in the art to prevent dimerization may be

used. A preferred multipeptide formulation includes at least one first peptide and at least one second peptide of ragweed pollen protein each having human T cell stimulating activity and soluble at a physiologically acceptable pH and selected from the group of peptides. In a preferred embodiment, the multipeptide formulation includes Peptides Amb 1-2.1, Amb 4-9.1, Amb 2-36.1, and modifications thereof, and sodium phosphate and mannitol. In this embodiment, it is preferred that Peptides Amb 1-2.1, Amb 4-9.1, and Amb 2-36.1 are in the form of a lyophilized powder which is reconstituted in a physiologically acceptable carrier, such as sterile water, prior to use. As an illustrative example, a multipeptide formulation comprising the three peptides were produced and used in Phase I human clinical trials (see Example XV). The peptides were combined during manufacturing to produce a vial containing a sterile, pyrogen free, lyophilized powder having the following composition:

**Active:** Peptide Amb 1-2.1, Peptide Amb 4-9.1, and Peptide Amb 2-36.1  
In concentration of 7.5-1500 µg per peptide

**Inactives:** 0.05 M Sodium Phosphate pH 7.5  
5% w/v Mannitol, U.S.P.

**Diluent:** Sterile Water for Injection, U.S.P. (initial reconstitution)  
0.9% Sodium Chloride for Injection  
(dilution beyond initial reconstitution)

The multipeptide formulation of the invention can be provided in the form of a kit, including instructions for use.

The present invention also provides methods of detecting sensitivity in individuals to ragweed pollen allergens comprising combining a blood sample obtained from the individual with a peptide of the present invention, under conditions appropriate for binding of blood components with the peptide and determining the extent to which such binding occurs. The extent to which binding occurs is determined by assessing T cell function, T cell proliferation or a combination thereof. Other diagnostic methods for allergic diseases which the protein or peptides of the invention can be used include radio-allergosorbent test (RAST), paper radioimmunosorbent test (PRIST), enzyme linked immunosorbent assay (ELISA), radioimmunoassays (RIA), immuno-radiometric assays (IRMA), luminescence immunoassays (LIA), histamine release assays and IgE immunoblots.

The presence in individuals of IgE specific for ragweed protein allergen and the ability of T cells of the individual to respond to T cell epitope(s) of the protein allergen can be determined by administering to the individuals an Immediate Type Hypersensitivity test and a Delayed Type Hypersensitivity test. The individuals are administered an Immediate Type Hypersensitivity test (see e.g. *Immunology* (1985) Roitt, I.M., Brostoff, J., Male, D.K. (eds), C.V. Mosby Co., Gower Medical Publishing, London, NY, pp. 19.2-19.18; pp. 22.1-22.10) utilizing purified native ragweed protein allergen, a peptide of ragweed protein, allergen or a modified form of the peptide, each of which binds IgE specific for the allergen. The same individuals are administered a Delayed Type Hypersensitivity test

prior to, simultaneously with, or subsequent to administration of the Immediate Type Hypersensitivity test. Of course, if the Immediate Type Hypersensitivity test is administered prior to the Delayed Type Hypersensitivity test, the Delayed Type Hypersensitivity test would only be given to those individuals exhibiting a specific 5 Immediate Type Hypersensitivity reaction. The Delayed Type Hypersensitivity test utilizes a modified form of ragweed pollen protein or a portion thereof, ragweed protein allergen produced by recombinant DNA techniques, or peptide derived from ragweed pollen protein, each of which has the ability to stimulate human T cells and each of which does not bind IgE specific for the allergen in a substantial percentage of the population of 10 individuals sensitive to the allergen (e.g., at least about 75%). After administration of the Delayed Type Hypersensitivity test, the extent to which a specific Delayed Type Hypersensitivity reaction occurs in the individual to the protein allergen or ragweed pollen protein peptide indicating presence in the individual of T cells specific to T cell epitope(s) of the protein allergen or ragweed pollen protein peptide is determined. Those individuals 15 found to have both a specific Immediate Type Hypersensitivity reaction and a specific Delayed Type Hypersensitivity reaction are diagnosed as having sensitivity to a ragweed allergen and may, if need be, administered a therapeutically effective amount of a therapeutic composition comprising the modified form of ragweed protein allergen or portion thereof, the ragweed protein allergen produced by recombinant DNA techniques. 20 or peptide, each as used in the Delayed Type Hypersensitivity test, and a pharmaceutically acceptable carrier or diluent.

It is also possible to modify the structure of a peptide of the invention for such purposes as increasing solubility, enhancing therapeutic or preventive efficacy, or stability (e.g., shelf life *ex vivo*, and resistance to proteolytic degradation *in vivo*.) A modified 25 peptide can be produced in which the amino acid sequence has been altered, such as by amino acid substitution, deletion, or addition, to modify immunogenicity and/or reduce allergenicity, or to which a component has been added for the same purpose.

For example, a peptide can be modified so that it maintains the ability to induce T cell 30 non-responsiveness and bind MHC proteins without the ability to induce a strong proliferative response or possibly, any proliferative response when administered in immunogenic form. In this instance, critical binding residues for the T cell receptor can be determined using known techniques (e.g., substitution of each residue such as, for example, with alanine and determination of the presence or absence of T cell reactivity). Those 35 residues shown to be essential to interact with the T cell receptor can be modified by replacing the essential amino acid with another, preferably similar amino acid residue (a conservative substitution) whose presence is shown to enhance, diminish, but not eliminate, or not affect T cell reactivity. In addition, those amino acid residues which are not essential for T cell receptor interaction can be modified by being replaced by another amino acid whose incorporation may enhance, diminish or not affect T cell reactivity, but not eliminate

binding to relevant MHC. Preferred amino acid substitutions for non-essential amino acids include, but are not limited to substitutions with alanine, glutamic acid or a methyl amino acid.

Another example of a modification of peptides is substitution of cysteine residues 5 preferably with serine, threonine, leucine or glutamic acid to minimize dimerization via disulfide linkages. As described in Example XI, a peptide from Region 2, RAE 70.1 (SEQ ID NO:44) was modified to minimize dimerization by substituting serine for the cysteine residue at amino acid position 212. In addition, as shown in Figure 23, peptide AMB 4-9.1 which contains an acid-sensitive aspartic acid-proline bond at amino acid residues 360-361, 10 was modified to increase the stability of this peptide. For example, in peptide AMB 4-9.1DA, the proline at position 361 was substituted with alanine to remove the acid-sensitive bond.

Stability may also be enhanced in those peptides shown to be susceptible to 15 degradation by deamidation (e.g. the peptide contains a labile Asn-Gly sequence susceptible to deamidation under various conditions. In such situations it has been found that lyophilization stabilized such peptides against deamidation. Peptide Amb 4-9.1 contained a labile Asn-Gly sequence and was found to be susceptible to deamidation under accelerated conditions (e.g. increased buffer concentration, increased ionic strength and increased temperature). Lyophilization stabilized the peptide against deamidation with no significant increase in 20 degradants following 6 months of storage at 5°C.

In order to enhance stability and/or reactivity, peptides can also be modified to incorporate one or more polymorphisms in the amino acid sequence of a protein allergen resulting from natural allelic variation. Additionally, D-amino acids, non-natural amino acids or non-amino acid analogues can be substituted or added to produce a modified peptide 25 within the scope of this invention. Furthermore, peptides can be modified using the polyethylene glycol (PEG) method of A. Sehon and co-workers (Wie et al., *supra*) to produce a peptide conjugated with PEG. In addition, PEG can be added during chemical synthesis of a peptide of the invention. Modifications of peptides or portions thereof can also include reduction/alkylation (Tarr in: *Methods of Protein Microcharacterization*, J.E. Silver ed., 30 Humana Press, Clifton, NJ, pp. 155-194 (1986)); acylation (Tarr, *supra*); esterification (Tarr, *supra*); chemical coupling to an appropriate carrier (Mishell and Shiigi, eds, *Selected Methods in Cellular Immunology*, WH Freeman, San Francisco, CA (1980); U.S. Patent 4,939,239); or mild formalin treatment (Marsh *International Archives of Allergy and Applied Immunology* 41: 199-215 (1971)).

35 In another embodiment, peptides within an allergen group (e.g., *Amb a I* or *Amb a II*) can be modified to enhance T cell reactivity. Given the cross-reactivity within the *Amb a I* family and *Amb a II*, a peptide of one group allergen which may be less reactive than a peptide of another group allergen corresponding in amino acid position can have one or more amino acids substituted with one or more amino acids from the corresponding peptide.

Additionally, peptides can be modified to incorporate a polymorphism in the amino acid sequence of a protein allergen resulting from natural allelic variation. Modification of peptides to include one or more of these polymorphisms may result in enhanced stability and/or reactivity.

5 To facilitate purification and potentially increase solubility of peptides of the invention, it is possible to add reporter group(s) to the peptide backbone. For example, poly-histidine can be added to a peptide to purify the peptide on immobilized metal ion affinity chromatography (Hochuli, E. et al., *Bio/Technology*, 6:1321-1325 (1988)). In addition, specific endoprotease cleavage sites can be introduced, if desired, between a reporter group  
10 and amino acid sequences of a peptide to facilitate isolation of peptides free of irrelevant sequences. In order to successfully desensitize an individual to a protein antigen, it may be necessary to increase the solubility of a peptide by adding functional groups to the peptide or by not including hydrophobic T cell epitopes or regions containing hydrophobic epitopes in the peptides. For example, in Region 3 of the *Amb a 1.1* protein, a selected peptide AMB 3-  
15 4.1 was modified to increase its solubility by the addition of three naturally occurring sequential residues found in the *Amb a 1.1* protein, "RHG", to the 5' end of the peptide. These residues are not necessary for T cell recognition and are also found in peptide AMB 3-  
20 5.1. Similarly, as described in Example XI, peptide RAE 70.1 was divided into two fragments and modified to increase solubility. As shown in Figure 25, a fragment of RAE 70.1 ("ORIGINAL" in Figure 25, amino acid residues 194-216, SEQ ID NO:125) was modified by substitution or addition of charged amino acids to increase the hydrophilicity and decrease the pI of the peptide to thereby increase the solubility. For example, in peptide Amb 2-22.1, isoleucine was substituted with glutamic acid to decrease the pI and avoid precipitation of the peptide from solution at a physiological pH. Similar substitutions and  
25 additions are shown in Figure 25. In addition, charged amino acids or charged amino acid pairs or triplets when added to the carboxy or amino terminus of the peptide may be particularly useful to increase the solubility of the peptide. Examples of charged amino acids include, but are not limited to arginine (R), lysine (K), histidine (H), glutamic acid (E), and aspartic acid (D). Examples of such modifications are shown in Fig. 14, AMB 2-8.1, AMB 2-9.1, AMB 2-10.1, AMB 2-7.1 and AMB 2-11.1.

30 To potentially aid proper antigen processing of T cell epitopes within a peptide, canonical protease sensitive sites can be recombinantly or synthetically engineered between regions, each comprising at least one T cell epitope. The resulting peptide can be rendered sensitive to cathepsin and/or other trypsin-like enzymes cleavage to generate portions of the peptide containing one or more T cell epitopes. In addition, such charged amino acid residues can result in an increase in solubility of a peptide.

35 Site-directed mutagenesis of DNA encoding a peptide of the invention can be used to modify the structure of the peptide. Such methods may involve PCR with degenerate oligonucleotides (Ho et al., *Gene*, 77:51-59 (1989)) or total synthesis of mutated genes

(Hostomsky, Z., et al., *Biochem. Biophys. Res. Comm.*, **161**:1056-1063 (1989)). To enhance bacterial expression, the aforementioned methods can be used in conjunction with other procedures to change the eucaryotic codons in DNA constructs encoding peptides of the invention to ones preferentially used in *E. coli*, yeast, mammalian cells or other eucaryotic cells.

Another aspect of the invention pertains to an antibody specifically reactive with *Amb a I*, or a fragment thereof. The antibodies of this invention can be used to standardize allergen extracts or to isolate the naturally-occurring or native form of *Amb a I*. For example, by using proteins or fragments thereof based on the cDNA sequence of *Amb a I*, anti-protein/anti-peptide antisera or monoclonal antibodies can be made using standard methods. A mammal such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of such protein or an antigenic fragment which is capable of eliciting an antibody response. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. *Amb a I* or fragment thereof can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibodies.

Following immunization, anti-*Amb a I* antisera can be obtained and, if desired, polyclonal anti-*Amb a I* antibodies isolated from the serum. To produce monoclonal antibodies, antibody-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Such techniques are well known in the art, for example the hybridoma technique originally developed by Kohler and Milstein, (*Nature* (1975) **256**:495-497) as well as other techniques such as the human B cell hybridoma technique (Kozbar et al., *Immunology Today* (1983) **4**:72) and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer Therapy* (1985) Alan R. Liss, Inc. pp. 77-96). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with *Amb a I* and the monoclonal antibodies isolated.

The term antibody as used herein is intended to include fragments thereof which are also specifically reactive with *Amb a I*. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example,  $F(ab')_2$  fragments can be generated by treating antibody with pepsin. The resulting  $F(ab')_2$  fragment can be treated to reduce disulfide bridges to produce  $Fab'$  fragments. The antibody of the present invention is further intended to include bispecific and chimeric molecules having an anti-*Amb a I* portion.

Another aspect of this invention provides T cell clones and soluble T cell receptors specifically reactive with *Amb a I* or a fragment thereof. Monoclonal T cell populations (i.e., T cells genetically identical to one another and expressing identical T cell receptors) can be

derived from an individual sensitive to *Amb a I*, followed by repetitive *in vitro* stimulation with *Amb a I* or portion thereof in the presence of MHC-matched antigen-presenting cells. Single *Amb a I* MHC responsive cells can then be cloned by limiting dilution and permanent lines expanded and maintained by periodic *in vitro* restimulation. Alternatively, *Amb a I* specific T-T hybridomas can be produced by a technique similar to B cell hybridoma production. For example, a mammal, such as a mouse can be immunized with *Amb a I* or fragment thereof, T cells from the mammal can be purified and fused with an autonomously growing T cell tumor line. From the resulting hybridomas, cells responding to *Amb a I* or fragment thereof are selected and cloned. Procedures for propagating monoclonal T cell populations are described in *Cellular and Molecular Immunology* (Abul K. Abbas et al. ed.), 10 W.B. Saunders Company, Philadelphia, PA (1991) page 139. Soluble T cell receptors specifically reactive with *Amb a I* or fragment thereof can be obtained by immunoprecipitation using an antibody against the T cell receptor as described in *Immunology: A Synthesis* (Second Edition), Edward S. Golub et al., ed., Sinauer Associates, 15 Inc., Sunderland, MA (1991) pages 366-269.

T cell clones specifically reactive with *Amb a I* or fragment thereof can be used to isolate and molecularly clone the gene encoding the relevant T cell receptor. In addition, a soluble T cell receptor specifically reactive with *Amb a I* or fragment thereof can be used to interfere with or inhibit antigen-dependent activation of the relevant T cell subpopulation, for 20 example, by administration to an individual sensitive to a cat allergen. Antibodies specifically reactive with such a T cell receptor can be produced according to the techniques described herein. Such antibodies can be used to block or interfere with the T cell interaction with peptides presented by MHC.

The present invention also provides nucleic acids having sequences encoding proteins and peptides of the invention. Nucleic acid sequences used in any embodiment of this 25 invention can be cDNA as described herein, or alternatively, can be any oligodeoxynucleotide sequence having all or a portion of a sequence represented herein, or their functional equivalents. Such oligodeoxynucleotide sequences can be produced chemically or mechanically, using known techniques. A functional equivalent of an 30 oligonucleotide sequence is one which is 1) a sequence capable of hybridizing to a complementary oligonucleotide to which the sequence (or corresponding sequence portions) of SEQ ID NO: 1, 3, 5, 7, 9 and 11 or fragments thereof hybridizes, or 2) the sequence (or corresponding sequence portion) complementary SEQ ID NO: 1, 3, 5, 7, 9 and 11 and/or 3) a sequence which encodes a product (e.g., a polypeptide or peptide) having the same functional 35 characteristics of the product encoded by the sequence (or corresponding sequence portion) of SEQ ID NO: 1, 3, 5, 7, 9 and 11. Whether a functional equivalent must meet one or more criteria will depend on its use (e.g., if it is to be used only as an oligoprobe, it need meet only the first or second criteria and if it is to be used to produce a peptide of the present invention, it need only meet the third criterion).

As described in the Examples which follow, *Amb a I* and *Amb a II* proteins have been recombinantly expressed in *E. coli*, purified and shown to have reduced binding to human allergic ragweed pollen IgE on Western blots. Overlapping peptides derived from the *Amb a I.1* protein and various peptides derived from *Amb a I.3* and *Amb a I.2* were synthesized and used to identify regions of T cell reactivity within the protein. These regions of T cell reactivity were further defined by modifying selected *Amb a I.1* peptides and determining T cell reactivity to these peptides.

This invention is further illustrated by the following non-limiting examples.

10 **Example I Native Ragweed Pollen Allergen Purification**

What follows is a description of the work done to biochemically purify the allergens of *Ambrosia artemisiifolia* in their native form as primary antigens for human T-cell epitope mapping.

15 50 g of defatted short ragweed pollen (Greer Labs) was extracted in 500 ml .05 M Tris pH 7.95 containing protease inhibitors. The extract was then depigmented by batch absorption with Whatman DE-52 DEAE cellulose (150 g dry weight) in the presence of 0.2 M NaCl at 4°C. Unbound material was dialysed against .025 M Tris pH 7.95 with protease inhibitors. The depigmented sample was next applied to an 80 ml DEAE cellulose column (Whatman DE-52) equilibrated in .025 M Tris pH 7.95 containing protease inhibitors.

20 Acidic proteins were eluted with .025 M Tris, 0.2 M NaCl pH 7.95 at 4°C with inhibitors.

In order to biochemically purify *Amb a I*, the acidic DEAE elution sample was fractionated by ammonium sulfate precipitation into 0-45% and 45-59% saturation samples (4°C). The *Amb a I*-enriched 45-59% pellet was applied at 0.5 ml/min (4°C) to a 500 ml Sephadryl S200 (Pharmacia) column in .05 M ammonium bicarbonate containing inhibitors.

25 Purified *Amb a I* was recovered in the 38 kD region and dialysed against .04 M Tris pH 8.0. This sample was next applied to an 8 ml Mono Q HR 10/10 (Pharmacia) column in .04 M Tris pH 8.0 at 25°C. Elution was performed with .04 M Tris pH 8.0 containing .08M NaCl and the major peaks were analyzed as discussed below for confirmation and purity of *Amb a I*.

30 To biochemically purify *Amb a II*, the acidic DEAE elution sample was separated into an *Amb a II*-enriched fraction by ammonium sulfate precipitation at 0-45% saturation (4°C). The pellet was applied at 0.5 ml/min (4°C) to a 200 ml Sephadex G75 (Pharmacia) column in .05 M ammonium bicarbonate containing inhibitors. Purified *Amb a II* was recovered in the 38 kD region and dialysed against .04 M Tris pH 8.0 at 25°C to separate contaminating *Amb a I* from *Amb a II*. Elution was performed with .04 M Tris pH 8.0 containing .08M NaCl.

The major peaks were analyzed by IEF SDS-PAGE using a mixture of 4.5-5.3 and 3.5-10.0 ampholytes (Pharmacia) in a 7% acrylamide gel. Protein sequencing was also performed to confirm *Amb a II*.

In another embodiment *Amb a* I.1 and *Amb a* I.2 were biochemically purified as follows. Defatted short ragweed pollen was purchased from Greer laboratories (Lenior, North Carolina). The pollen (50g) was extracted overnight at 4°C with extraction buffer (10ml/g pollen) containing 50 mM Tris-HCl, pH8 and protease inhibitors in final 5 concentrations: phenyl methyl sulfonyl fluoride (170 µg/ml); soybean trypsin inhibitor (1µ g/ml); leupeptin (1 µg/ml) and peptstatin A (1 µg/ml). The soluble extract was clarified by sequential filtration through Whatman #1 paper (Whatman, Maidstone England) followed by an 0.8 micron filter. The soluble pollen extract (SPE) was either used for IgE binding studies or subjected to further purification as described below. For purification of *Amb a* I.1 and 10 *Amb a* I.2, sodium chloride was added to SPE to a final concentration of 0.3M and the material was depigmented by batch absorption onto What DE 52 cellulose (50 g dry weight) with extraction buffer plus 0.3 M NaCl. The solution was separated from the resin by filtration (Whatman #1 paper, Whatman, Maidstone England). The sample was then 15 fractionated by sequential (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitation, first at 0-47.5% and then at 47.5%-60% saturation at 4°C. The latter precipitate enriched in *Amb a* I, was separated by centrifugation (10,000 x g, 1h) and the pellet was resuspended in 20 mM Tris-HCl, pH 8. The sample was then loaded onto an anti-*Amb a* I murine monoclonal antibody affinity 20 column (mAb-4B5B7, Dr. D. Klapper, Univ. North Carolina, Chapel Hill, NC) which was determined to bind members of the *Amb a* I/*Amb a* II family. *Amb a* I was eluted with 0.1 M glycine, pH11 and was immediately neutralized with 1 M sodium phosphate, pH 3.9. This preparation contained both *Amb a* I.1 and *Amb a* I.2 as detected by SDS-PAGE analysis and by Western blotting. The affinity purified proteins were pooled, concentrated and dialyzed against 20 mM Tris, pH 8.5, extensively. *Amb a* I.1 and *Amb a* I.2 were further separated by 25 a Source Q ionic exchange column (Pharmacia, Piscataway, NJ), which was equilibrated with 20 mM Tris, pH 8.5. The flow-through material contained *Amb a* I.2 whereas *Amb a* I.1 was eluted with step gradients of 24 mM and 80 mM NaCl. The latter peak also contained some α,β cleaved fragments of *Amb a* I.1. *Amb a* I.1 and *Amb a* I.2 purity was verified by NH<sub>2</sub>-terminal amino acid sequencing as being greater than 95%.

### 30 Example II Recombinant Ragweed Pollen Allergen Expression

What follows is a description of the work done to produce the allergens of *Ambrosia artemisiifolia* as recombinant proteins in *E. coli*.

Described and provided in USSN 07/529,951, filed May 29, 1990, (incorporated herein by reference) are full length cDNAs encoding *Amb a* I.1, *Amb a* I.2, *Amb a* I.3, *Amb a* I.4 and *Amb a* II. cDNA inserts encoding these five protein allergens were constructed in the 35 vector pTrc99A (Amann, E.. et al., *Gene*, 69:301 (1988)) which was kindly provided by Dr. Egon Amann (Behringwerke AG, Marburg, FRG). The nucleotide sequences and deduced amino acid sequences of the *Amb a* I family members are shown in the sequence listing as SEQ ID NO:1 and 2 (*Amb a* I.1), SEQ ID NO:3 and 4 (*Amb a* I.2), SEQ ID NO:5 and 6

(*Amb a* I.3), SEQ ID NO:7 and 8 (*Amb a* I.4), and SEQ ID NO:9 and 10 (*Amb a* II). The cDNAs encoding each allergen were cloned in frame with a polylinker encoding six sequential histidines, (CAC)<sub>6</sub>, that had been inserted into the 5' end of the pTrc99A vector as a NcoI/EcoRI synthetic adapter (Maniatis T., et al.

5 *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). These cDNA inserts were then cloned in-frame into the appropriate pTrc99. To further enhance expression, a retroregular stem-loop sequence was placed at the 3' end in the untranslated region (Skoglund, C. M., et al. *Gene* 88:1 (1990)). The H<sub>6</sub> leader sequence allowed purification using QIAGEN NTA-Agarose (Diagen GmbH, Dusseldorf, 10 FRG), a Ni<sup>2+</sup> chelating support (Hochuli, E. et al., *Biotechnology*, 6:1321 (1988)). The vectors were transformed into the XL-1 Blue host bacteria. Expression of individual recombinant proteins was induced when cultures reached OD<sub>600</sub>=0.6 to 0.7 by the addition 15 of isopropyl-D-thiogalactopyranoside (IPTG) to 1 mM concentration to the culture medium. After 2 hours of further growth at 37°, the cells were pelleted. Recombinant proteins were then obtained by either of the following procedures. In one embodiment, the cells were 20 resuspended in lysozyme containing phosphate buffer (0.4 mg/ml) and incubated for 30 minutes on ice. The cell suspension was frozen and quick thawed followed by sonication (Bond, J. G. et al., *J. Immunol.*, 146:3380 (1991)). Insoluble recombinant protein was recovered by a low speed centrifugation and solubilized in 10 ml (per 1 liter growth) of 25 buffer containing 8 M urea, 50 mM Tris, pH 8.0, 2 mg/ml leupeptin, 2 mg/ml pepstatin and 1 mg/ml soybean trypsin inhibitor. The urea solubilized preparation was subjected to a low speed centrifugation and the recombinant proteins in the supernatant isolated by metal ion chromatography (Hochui *supra*).

30 In another embodiment, the pelleted bacteria were resuspended in 6 M guanidine HC1, 100 mM 2-mercaptoethanol, 100 mM NaPO<sub>4</sub>, 10 mM Tris pH 8.0. This suspension was subjected to centrifugation at 15,000 X g, and the supernatant removed, adjusted to pH 8.0 with 10 N NaOH, and applied to an NTA agarose column that had been equilibrated in 6 M guanidine HC1, 100 mM NaPO<sub>4</sub>, 10 mM Tris pH 8.0. The column was washed in 6 M guanidine HC1, 100 mM NaPO<sub>4</sub>, 10 mM Tris pH 8.0 until the OD<sub>280</sub> of the effluent reached background. The column buffer was then switched to 8 M urea, 100 mM NaPO<sub>4</sub>, 10 mM Tris pH 8.0. After equilibration, a more stringent wash was performed in 8 M urea, 100 mM NaOAc, 10 mM Tris pH 6.3 until the OD<sub>280</sub> of the effluent reached background. Recombinant protein (as an H<sub>6</sub> fusion) was then eluted in 8 M urea, 100 mM NaOAc, 10 mM Tris pH 4.5 and collected in aliquots whose OD<sub>280</sub> profile was monitored. The protein 35 peak was dialyzed 3 times into 500 volumes of PBS for human T-cells analysis. Yield ranged from 1-3 mg of recombinant protein per litre with purity of approximately 55% (as determined by densitometric scanning).

In another embodiment, high yields (e.g. 50-100 mg purified r *Amb a* I.1 protein/L of fermentation broth) were recovered. This embodiment involves the use of a construct which

is different from the one described above in 3 ways: 1)deletion of sequence coding for 36 non-*Amb a I* related amino acids, 2) attachment of a 6 histidine linker to the NH<sub>2</sub>-terminus, and 3) replacement of 2 existing arginine codons with *E. coli*-preferred arginine codon. The resulting construct showed significantly higher expression levels than did the original construct. This phenomenon was true in both shaker flask and 10L fermentation cultures. The purification also differed significantly from the procedure described above. Cell paste from fermentors was homogenized and inclusion bodies were purified prior to solubilization in guanidine-HCL. The solubilized inclusion bodies were then purified using metal chelate chromatography and buffer exchanged into acetate buffer. The resulting protein is greater than 90% full length r *Amb a I*.1.

### Example III IgE Analysis With Purified Recombinant Ragweed Pollen Allergens

Both Western blotting and ELISA techniques were used to analyze the binding of IgE to purified recombinant *Amb a I* and *Amb a II* proteins. Additionally, ELISA was used for analysis of IgE reactivity to peptides derived from the *Amb a I*.1 protein.

#### A. Western Blot Analysis

The antigens were loaded on the gels as follows: lane 1 SPE (Soluble Pollen Extract), 15 mg/lane; lane 2 r*Amb a I*.1 (recombinant *Amb a I*.1), 3 mg/lane; lane 3 r*Amb a I*.2, 4 mg/lane; lane 4 r*Amb a I*.3, 3 mg/lane; lane 5 r*Amb a I*.4, 3 mg/lane; and lane 6 r*Amb a II*.1, 4 mg/lane.

The gel electrophoresis and Western blot transfer procedures used were essentially as described elsewhere (Towbin, H., et al., *Proc. Natl. Acad. Sci., USA*, **76**:4350 (1979)). Briefly, SDS-PAGE was performed in 10% acrylamide gels under reducing conditions (10mM dithiothreitol, at constant current). The transfer to nitrocellulose (0.1 mM, Schleicher & Schuell, Keene, NH) was performed in a Hoeffer apparatus according to the protocol of Towbin et al., *supra*. After transfer, the blots were rinsed in blot solution (25 mM Tris-HCl, pH 7.5, 0.17 M NaCl, and 0.05% Tween 20) and stained for 1 hour with 0.1% India ink. All subsequent incubations with antibodies and washes were performed in blot solution at room temperature. The first antibody incubations were performed overnight then rinsed and incubated with the appropriate biotinylated second antibody (Kirkegaard Perry Laboratories, Gaithersburg, MD). The final incubation was performed using <sup>125</sup>I streptavidin (1 mCi/25 ml blot solution) for 1 hour followed by removal of unbound labeled material and autoradiography at -80°C with an intensifying screen.

Fig. 1 shows the Western blot IgE binding pattern of two ragweed allergic patient plasma samples (#475 and #143). These patterns are representative of the typical IgE reactivity to these proteins on a Western blot. Both patients show binding to the *Amb a I*.1 and *Amb a I*.3 gene products in the soluble pollen extract (lane 1, Fig. 1). The results

demonstrate clear IgE binding reactivity in these patients to *Amb a* I.1, *Amb a* I.3, *Amb a* I.4. In contrast, *Amb a* I.2 and *Amb a* II.1 showed markedly reduced IgE binding.

### B. ELISA

5 Patient #143 IgE was also tested for binding to purified recombinant *Amb a* I and *Amb a* II proteins by ELISA. Fig. 2 shows the results of this analysis performed according to the following method.

10 Corning assay plates (#25882-96) were coated with each antigen listed in Fig. 2 at the following concentrations: Soluble Pollen Extract (SPE) 15 mg/ml; r*Amb a* I.1, 5 mg/ml; r*Amb a* I.2, 20 mg/ml; r*Amb a* I.3, 5 mg/ml; r*Amb a* I.4, 15 mg/ml; and r*Amb a* II, 20 mg/ml. 50 mls/well of the above antigens were added and coating was carried out overnight at 4° C. The coating antigens were removed and the wells were blocked with 0.5% gelatin in PBS, 200ml/well for 2 hours at room temperature. Patient #143 plasma was serially diluted with PBS-Tween 20 (PBS with 0.05% nonionic detergent Tween-20 Sigma, St. Louis MO) and 15 100ml/well was added and incubated overnight at 4°C (plasma dilutions are tested in duplicate). The second antibody (biotinylated goat anti-Human IgE, 1:1000, Kirkegaard & Perry Laboratories Inc. Gaithersburg, MD), was added at 100 ml/well for one hour at room temperature. This solution was removed and streptavidin-HRPO, 1:10000, (Southern Biotechnology Associates, Inc., Birmingham, AL) was then added at 100ml/well for one 20 hour at room temperature (all wells are washed three times with PBS-Tween between each incubation step). TMB Membrane Peroxidates Substrate system (Kirkegaard & Perry Laboratories) was freshly mixed, and added at 100ml/well. The color was allowed to develop for 2-5 minutes. The reaction was stopped by the addition of 100ml/well of 1 M phosphoric acid. Plates were read on a Microplate EL 310 Autoreader (Biotek Instruments, 25 Winooski, VT) with a 450nm filter. The absorbance levels of duplicate wells was averaged. The graphed results (log of the dilution vs absorbance) of the ELISA assays are shown in Fig. 2. The order of coating antigens listed vertically in these figures corresponds in order from left to right to the coating antigens listed for each histogram.

30 The results of the ELISA assay, Fig. 2, demonstrate a similar reactivity pattern as seen in the Western blot analysis (Fig. 1). The observation of much stronger binding to SPE is due to the fact that this antigen preparation is non-denatured. However, the recombinant *Amb a* proteins, because of their non-native bacterial origin behave as denatured antigens. The hierarchy of IgE binding in this patient to r*Amb a* proteins by ELISA shows that *Amb a* I.1 binds IgE more strongly than *Amb a* I.3 and *Amb a* I.4. The results also show that *Amb a* I.2 and *Amb a* II demonstrate markedly reduced binding to IgE.

35 As shown in Fig. 3, another ELISA assay was performed using a pool of human allergic sera. This assay demonstrates that biochemically purified (native) *Amb a* I and *Amb a* II proteins bind significantly more IgE than the respective recombinant proteins (r*Amb a* I.1 and r*Amb a* II). (The control in this experiment was plates coated without antigen and PBS).

C. Modified ELISA for Greater Sensitivity

In another embodiment, an ELISA based assay system for serum IgE binding to peptides was developed which had greater sensitivity than direct ELISA, and creating tandem 5 *Amb a 1* peptide copies in the form of a recombinant trimer. One strategy for constructing a recombinant trimer based on *Amb a 1* peptide Amb 4-9.1 is as follows.

DNA sequences encoding Amb 4-9.1 are amplified by PCR from a plasmid containing *Amb a 1.1* sequence. Three Amb 4-9.1 copies are made each containing unique 10 restriction enzyme cleavage sites. The restriction sites are designed (inserted during the amplification reaction) to ensure peptide ligation to each other or ligation to plasmid. Amb 4-9.1 copies are ligated together and then the fused sequences are ligated into a sequencing vector, pUC19. After DNA sequence confirmation, the trimer sequences are excised from pUC19 and inserted into an expression vector pEt-11d containing a 6 histidine tag.

After DNA sequence confirmation, the expression plasmid containing Amb 4-9.1 15 trimer sequence was expressed in *E.coli* BL21. Expressed protein was purified over a  $\text{Ni}^{2+}$  column, protein was analyzed by SDS-Page, Western, ELISA and protein sequencing. The Amb 4-9.1 trimer was greater than 95% pure and was soluble in PBS. The sequence of the Amb 4-9.1 trimer was confirmed as:

MGHHHHHHHEFELGTVLENGAIFVASGVDPVLTPEQSAGSRGK  
20 DVLENGAIFVASGVDPVLTPEQSAGLQGTVLENGAIFVASGVDPVLTPEQSAG.

Side by side direct ELISAs were carried out in order to compare the sensitivity of assays using unmodified Amb 4-9.1 peptide versus recombinant Amb 4-9.1 trimer 25 constructed as discussed above. These assays were carried out using a panel of plasma samples from individuals with high skin test reactivity to *Amb a 1*. Plasma previously shown to have IgE binding to unmodified Amb 4-9.1 peptide served as a positive control. When IgE binding to the Amb 4-9.1 recombinant trimer was detected, competition ELISA was used to verify that the binding was relevant to the unmodified Amb 4-9.1 peptide (rather than to a novel epitope created by the recombinant Amb 4-9.1 structure).

There was a significant enhancement of assay sensitivity (both the number of 30 positives and assay signal/noise ratio) using the Amb 4-9.1 recombinant trimer. Thus, a very sensitive assay has been developed which is useful for screening IgE binding to any *Amb a 1* pollen protein allergen peptide. This assay is particularly useful for screening *Amb a 1.1* peptides which are to be formulated into a human therapeutic. This modified assay is also 35 useful for screening for IgE binding to any peptide derived from any antigen by first constructing a recombinant trimer of the peptide of interest as discussed above and using the recombinant trimer in the assay for detecting IgE binding.

**Example IV Tolerization of Mice with Recombinant *Amb a I* Proteins**

Balb/c mice (H-2 d) were immunized in the hind foot pads and at the base of the tail with an emulsion of Complete Freunds Adjuvant (CFA) and 50mg/mouse *Amb a I.1*. Seven days later, the popliteal lymph nodes, superficial inguinal lymph nodes, and the periaortic lymph nodes were harvested and cultured ( $1 \times 10^5$  lymph node cells +  $2 \times 10^5$  irradiated spleen feeders) with various challenge antigens *in vitro*. The *in vitro* antigens consisted of various doses of *Amb a I.1*, *Amb a I.2*, *Amb a I.3*, *Amb a I.4*, *Amb a II*, and a media control. Cultures were incubated for 3 days at 37°C in a CO<sub>2</sub> incubator. On day 3, each culture was pulsed with 1mCi <sup>3</sup>H thymidine and on day 4 the cultures were harvested and proliferation was monitored by incorporation of <sup>3</sup>H into DNA.

As seen in Fig. 4A, the mice that were immunized with 50mg *Amb a I.1* + CFA have a good response to *Amb a I.1*, *Amb a I.3*, and *Amb a I.4*. The response to *Amb a I.2* and *Amb a II* is also good but less than other antigens. It appears as though *Amb a I.1* is very immunogenic in Balb/c mice and results in a response to each *Amb a* family member. When mice were immunized with PBS + CFA, there was no significant response to any of the *Amb a* family members (Fig. 4B).

An experiment was conducted to determine whether mice could be tolerized with *Amb a I.1*. The mice were tolerized with *Amb a I.1* and pollen extract and then challenged with *Amb a I.1* or pollen extract. The outline of this experiment #5 is shown below:

20 Day 1:      Group (1): 6 Balb/c mice tolerized with 300mg *Amb a I.1* + Incomplete Freunds Adjuvant (IFA) intraperitoneally (i.p.)  
                  Group (2): 6 Balb/c mice tolerized with 300mg Pollen Extract + IFA i.p.  
                  Group (3): 6 Balb/c mice exposed to PBS + IFA i.p.

25 Day 15      Challenge 3 mice from each group with 50mg *Amb a I.1* + CFA subcutaneously (s.c.)  
                  Challenge the remaining 3 mice from each group with 50mg Pollen extract + CFA s.c.

30 Day 23      Harvest Lymph Nodes (Analyze each mouse separately)  
                  Test Lymph Node Response on *Amb a I.1*, *I.2*, *I.3*, *I.4*, *II.1*, Pollen Extract, Concanavalin A (Con A), and media.

35              The animals were sacrificed by cervical dislocation on day 23 and the popliteal lymph nodes, superficial inguinal lymph nodes, and the periaortic lymph nodes were removed and placed in rinsing buffer (cold RPMI 1640 containing 1% FCS). The nodes were rinsed with rinsing buffer and forced through a fine stainless steel mesh, using a glass pestal to suspend them in rinsing buffer. The suspended cells were rinsed two times by centrifugation at 1000

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rpm for 10 minutes and resuspended in rinsing buffer. An aliquot from each sample was taken in order to do a cell count. The cells ( $4 \times 10^6$ /ml) were incubated in culture media (RPMI 1640 media containing 10% FCS, 2mM L-glutamine, 50U/ml penicillin, 50 mg/ml streptomycin and  $5 \times 10^{-5}$  M 2-mercaptoethanol) and test antigens at various concentrations.

5 The triplicate 0.1 ml cultures (U-bottom 96 well plates (Costar)) were incubated at 37°C and at 5% CO<sub>2</sub>. After 24 hours, 50ml of media from each well was placed in separate flat bottom 96 well plates (Costar) and frozen overnight at -20°C to eliminate carryover of live cells.

10 The supernatants were tested after thawing for their ability to support the growth of CTL3, an IL-2 dependent T cell clone. CTL3 in log phase growth were rinsed 3 times by centrifugation at 1000 rpm for 10 minutes. CTL3's were added to the warmed culture supernatants ( $5 \times 10^3$  cells/well) and the IL-2 assay was incubated at 37°C and 5% CO<sub>2</sub>. After 24 hours, 1 mCi/well <sup>3</sup>H thymidine was added in 50 ml/well and the CTL3 cells were incubated for an additional 4-6 hours. Following the pulse with <sup>3</sup>H, the plates were

15 harvested on a Tom Tek 96 well cell harvester. The <sup>3</sup>H incorporation in each well was counted by a Betaplate Model 1205 scintillation counter. Background counts were not subtracted.

Fig. 5 shows the lymph node responses to *Amb a 1.1*, *Amb a 1.2*, *Amb a 1.3*, *Amb a 1.4*, and *Amb a II* in the mice that were tolerized with *Amb a 1.1* or Phosphate Buffered Saline (PBS) and challenged with *Amb a 1.1*. Fig. 5A, shows that the mice that were tolerized with *Amb a 1.1* have a lower response to *Amb a 1.1* than the mice that were tolerized with PBS. The same appears to be true in the response to *Amb a 1.2*, *Amb a 1.3*, and *Amb a 1.4* (Figs. 5B, 20 5C, 5D). In Figs. 5E and 5F, it appears as though there is no significant difference in the response to *Amb a II* or pollen extract between the tolerized group and the non-tolerized group. Figs. 6A-6F show the lymph node responses to the various proteins in the mice that 25 were exposed to pollen extract or PBS and challenged with pollen extract. Table 1 summarizes the results of these experiments.

TABLE 1

	<u>Amba 1.1</u>	<u>Amba 1.2</u>	<u>Amba 1.3</u>	<u>Amba 1.4</u>	<u>Amba II.1</u>	<u>Pollen</u>
30 Tolerize/Challenge						
Control/ <u>Amba 1.1</u>	+	+	+	+	-	-
<u>Amba 1.1/Amba 1.1</u>	-	-	-	-	-	-
35 Control/Pollen	+	+	+/-	+/-	-	+
Pollen/Pollen	-	-	-	-	-	-

(-) indicates a diminished response as compared to control

**Example V Synthesis of Overlapping Peptides**

*Amb a I.1* overlapping peptides as shown in Fig. 7 were synthesized using standard Fmoc/tBoc synthetic chemistry and purified by dialysis or Reverse Phase HPLC. In addition, various peptides derived from *Amb a I.2* and *Amb a I.3* were synthesized. The amino acid residues of the synthesized peptides are in brackets by the peptide name and the amino acid sequence (in single letter code) is next to the peptide name. The peptide names are consistent throughout the Figures. In the design of the overlapping peptides, the relationship of the *Amb a I* family members at the level of T cell cross-reactivity as determined in Example VIII was considered. As shown in Table IV, the *Amb a I* protein allergens share a high degree of cross-reactivity. In addition, *Amb a I.1* and *Amb a II* were found to have 55.2% cross-reactivity. Given the high degree of cross-reactivity it was expected that "shared" T cell epitopes exist among Groups I and II. Thus, the amino acid sequences of the *Amb a I* family members and *Amb a II* were examined to identify conserved and variable regions. It was expected that conserved regions within the *Amb a I* family members and *Amb a II* would contain "shared" T cell epitopes.

**Example VI T Cell Responses to Ragweed Peptides**

Peripheral blood mononuclear cells (PBMC) were purified by Sepracell-MN or lymphocyte separation medium (LSM) centrifugation of 60 ml of heparinized blood from 20 ragweed-allergic patients who exhibited clinical symptoms of seasonal rhinitis and were skin prick test positive for ragweed. T cell lines were established from these cells by stimulation of  $1\cdot2 \times 10^6$  PBMC/ml in RPMI-1640 containing 5% human AB serum (complete medium) with recombinant *Amb a I.1* at 20-30 mg/ml for 5-6 days at 37°C in a humidified CO<sub>2</sub> incubator. Viable cells were purified by LSM centrifugation and cultured in complete 25 medium supplemented with 5 units recombinant human IL-2/ml and 5 units recombinant human IL-4/ml for up to three weeks until the cells no longer responded to lymphokines and were considered "rested". The ability of the T cells to proliferate to *Amb a I.1*, *Amb a I.2* and *Amb a I.3* sequence-derived synthetic peptides was then assessed.

For assay,  $2 \times 10^4$  rested cells were restimulated in the presence of  $2 \times 10^4$  30 autologous Epstein-Barr virus (EBV)-transformed B cells (gamma-irradiated with 25,000 RADS) or  $5 \times 10^4$  autologous PBMC (3,500 RADS) with various concentrations of *Amb a I.1* synthetic peptides in a volume of 200 ml complete medium in duplicate or triplicate wells in 96-well round bottom plates for 3 days. Each well then received 1 mCi tritiated thymidine for 16-20 hours. The counts incorporated were collected onto glass fiber filter mats and 35 processed for liquid scintillation counting. Table II shows the results of a representative assay. The maximum response in a titration of each peptide is expressed as the S.I. or stimulation index. The S.I. is the CPM incorporated by cells in response to peptide divided by the CPM incorporated by cells in medium only. An S.I. value greater than the background level is considered "positive" and indicates that the peptide contains a T cell epitope.

However, only individual S.I. values above 2.0 (a response two-fold or greater over background) were used in calculating mean stimulation indices for each peptide for the group of patients tested. The results shown in Table II demonstrate that this patient (#466) responds very well to peptides RAE 7.D, RAE 69.1, RAE 64.1, and RAE 29.1. This indicates that these peptides contain *Amb a* I.1 T cell epitopes recognized by T cells from this particular allergic patient.

TABLE II

10	Antigen	Concentration (mg/ml)	CPM	S.I.
	Medium	--	1677	--
	Amb a I.1	2	12113	
		10	25047	
15		50	45710	27.3
	RAE 22.E	5	3198	
		50	3581	2.1
20	RAE 7.D	5	3948	
		50	8572	5.1
	RAE 45.15	5	1160	
		50	1370	0.8
25	RAE 69.1	5	6800	
		50	11464	6.8
	RAE 70.1	5	4116	
30		50	3888	2.5
	RAE 65.1	5	1236	
		50	1784	1.1
35	RAE 64.1	5	12828	7.7
		50	2259	
	RAE 29.1	5	6998	
		50	13078	7.8

The above procedure was followed with a number of other patients. Individual patient results were used in calculating the mean S.I. for each peptide if the patient responded to the *Amb a I.1* protein at an S.I. of 2.0 or greater and the patient responded to at least one peptide derived from *Amb.a I.1* at an S.I. of 2.0 or greater. A summary of positive-  
5 experiments from 39 patients is shown in Fig. 8. The bar represents the cumulative rank of the peptide response in the 39 patients. To determine the cumulative rank, the 5 peptides with the highest S.I. in each patient were determined and assigned a numerical rank in descending order with 5 representing the strongest response. The ranks for each peptide were then summed in the 39 patients to determine the cumulative rank for the peptide. The  
10 number above each bar is the mean S.I. of the positive responses (S.I. of 2.0 or greater) from the group of patients to that peptide. In parentheses above each bar is the positivity index (P.I.). The P.I. for each peptide is determined by multiplying the mean S.I. by the percent of patients who responded to that peptide. The P.I. therefore represents both the strength of the response (S.I.) and the frequency of a response to a peptide in the group of patients tested.  
15 For example, peptide RAE 69.1 had the highest cumulative rank so it was the best peptide response in the overall population of 39 even though it did not have the highest mean S.I. Similarly, RAE 70.1 had the highest mean S.I but not the best cumulative rank or P.I. Thus, the response to RAE 70.1 was strong when it occurred but it did not occur as frequently in the population as the response to other peptides. The peptide with the highest P.I., RAE 29.1,  
20 also had a strong S.I. and the second highest cumulative rank. The response to this peptide was therefore generally strong and relatively frequent in this population.

#### Example VII T Cell Epitope Fine Map Studies with *Amb a I.1*

Based on the above analysis 4 major areas of T cell reactivity within *Amb a I.1* were identified. (Regions 1, 2, 3 and 4). All 39 patients responded to *Amb a I.1* and a peptide from at least one of these regions: Region 1, amino acids residues 48-107; Region 2, amino acid residues 171-216; Region 3, amino acid residues 278-322; and Region 4, amino acid residues 331-377. Based in part on the T cell reactivity shown in Fig. 8, *Amb a I.1* peptides were selected and modified by addition or deletion of amino acid residues at either the 5' or 3'  
30 end of the peptide. This set of peptides is shown in Fig. 9. T cell studies similar to those of Example VI were performed using these selected peptides to more precisely define the major areas of T cell reactivity within Regions 1-4 of the *Amb a I.1* protein. For example, PBMC from a single ragweed-allergic patient were isolated as described in Example VI and were stimulated with 20 mg/ml of recombinant *Amb a I.1* as described above. The results of  
35 proliferation assays with this one patient to selected peptides using irradiated (24,000 RADS) autologous EBV B cells as antigen presenting cells is shown in Table III. The data indicates that T cells from this patient respond well to RAE 7.D, RAE 70.1, RAE 40.1-4, and RAE 28.1-2.

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TABLE III

<u>Antigen</u>	<u>Concentration (mg/ml)</u>	<u>CPM</u>	<u>S.I.</u>
5 <b>Medium</b>	--	2762	--
10 <b>PHA</b>	1	97864	35.4
15 <b>Amb a 1.1</b>	2	28025	
	10	56172	
	50	86598	31.4
20 <b>RAE 22.E</b>	5	3318	1.2
	50	784	
25 <b>RAE 7.D</b>	5	53292	
	50	60943	22.1
30 <b>RAE 40.1-4</b>	5	4504	
	50	13549	4.9
35 <b>RAE 69.1</b>	5	5213	
	50	5981	2.2
40 <b>RAE 70.1</b>	5	14223	
	50	4729	5.1
45 <b>RAE 64.1</b>	5	3418	1.2
	50	2538	
50 <b>RAE 28.1-2</b>	5	7542	
	50	24208	8.8

The above procedure was followed with a number of other patients and yielded 47 positive experiments. A summary of the results for Region 1 peptides of the *Amb a I.1* protein is shown in Fig. 10, Region 2 peptides in Fig. 11, Region 3 peptides in Fig. 12, and Region 4 peptides in Fig. 13. As described in Example VI, the bar shows the cumulative rank. In these experiments the 3 best peptide responses were ranked. The number above each bar represents the mean S.I. for the peptide and the number in parentheses the P.I. for the peptide. In these experiments an individual S.I. of 2.5 or greater was used in calculating the mean S.I.

Fig. 10 indicates that the major area of T cell reactivity within Region 1 of the *Amb a I.1* protein is represented by peptides RAE 6.D, RAE 7.D, RAE 40.1, RAE 40.1-4, and RAE 40.1-5. Thus, a preferred area of major T cell reactivity within Region 1 comprises amino acid residues 57-101. Fig. 11 indicates that there is a broad area of weak T cell reactivity in Region 2 of the *Amb a I.1* protein relative to Region 1. A preferred area of major T cell reactivity within Region 2 thus comprises amino acid residues 182-216. Fig. 12 shows that the most frequent and dominant response within Region 3 of the *Amb a I.3* protein is to peptide RAE 64.1. However, another area of T cell reactivity is represented by peptide RAE 66.1. A preferred area of major T cell reactivity within Region 3 comprises amino acid residues 280-322. Fig. 13 indicates that the major area of T cell reactivity in Region 4 is represented by peptides RAE 28.1-2, RAE 28.1-1, and RAE 28.1. From this analysis, a preferred area of major T cell reactivity within Region 4 comprises amino acid residues 342-377.

To further validate and define peptides derived from the *Amb a I.1* protein comprising T cell epitopes, selected peptides from Regions 1-4 were further modified by addition or deletion of amino acid residues as described above. These selected modified peptides are shown in Fig. 14. To determine the T cell reactivity of these peptides, PBMC were stimulated with recombinant *Amb a I.1* and assayed with the selected peptides as described in Example VI, except in some cases autologous PBMC (irradiated 3,500 RADS) were used as antigen presenting cells. The assay was followed with a number of patients and resulted in 23 positive experiments. In these assays an individual S.I. of 2.0 was used in calculating the mean stimulation index. In Figs. 15-18, the bar represents the cumulative rank of each peptide. The best 3 peptide responses for each patient were ranked as described above. The mean S.I. for each peptide and percent positive are found above the bar.

#### Example VIII *Amb a I.1* Specific T Cell Cross-Reactivity To Other *Amb a I* Family Members and *Amb a II*

To determine the relationship of the *Amb a I* family members (i.e., *Amb a I.1*, *I.2*, *I.3*, and *I.4*) at the level of T cell reactivity, PBMC were stimulated with recombinant *Amb a I.1* (r *Amb a I.1*) as described in Example VI. For assay,  $2 \times 10^4$  rested cells were restimulated in the presence of an equal number of autologous EBV-transformed B cells (irradiated with

25,000 RADS) with various concentrations (0-100 mg/ml) of recombinant *Amb a* I.1, I.2, I.3, I.4, *Amb a* II, or ragweed pollen extract in a volume of 200 ml complete medium in duplicate or triplicate wells in 96-well round bottom plates for 3 days. Each well then received 1 mCi tritiated thymidine for 16-20 hours. The counts incorporated were assessed and analyzed as described in Example VI. Table IV shows the S.I., P.I., percent of cultures positive for the assay antigen, and number of cultures analyzed. For these assays an individual S.I. greater or equal to 2.0 was used in calculating the mean S.I. *Amb a* I.1 stimulated cells respond less well and less frequently to the least homologous family members I.3 and I.2. However, this level of cross-reactivity, 68.8% and 60% respectively, is still considered high. The lowest level of *Amb a* I.1 stimulated T cell cross-reactivity is noted to the least homologous allergen, *Amb a* II. In addition, *Amb a* I.1 reactive T cells are found at a high percentage from ragweed pollen extract stimulated cultures, again demonstrating the importance of the *Amb a* I.1 allergen.

15

TABLE IV

	<u>Assay Antigen</u>	Recombinant <i>Amb a</i> I.1			
		<u>Specific Lines</u>			
20		<u>S.I.</u>	<u>% positive</u>	<u>n</u>	<u>P.I.</u>
	Pollen Extract	13.5	87.8%	41	1185
25	r <i>Amb a</i> I.1	27.5	95.1%	41	2616
	r <i>Amb a</i> I.2	5.0	60%	5	300
	r <i>Amb a</i> I.3	9.3	68.8%	16	639
30	r <i>Amb a</i> I.4	8.5	100%	11	850
	r <i>Amb a</i> II	12.8	55.2%	29	70

**Example IX T Cell Cross-Reactivity of *Amb a I.1* or *Amb a I.3* Stimulated Cells to *Amb a I.1* or *Amb a I.3* Peptides**

Since a high degree of T cell cross-reactivity could be demonstrated between *Amb a I.1* and the most highly sequence divergent family member *Amb a I.3*, the ability of *Amb a I.1* 5 stimulated cells to recognize both *Amb a I.1* peptides and homologous *Amb a I.3* peptides was assessed. In a similar fashion, the ability of *Amb a I.3* stimulated cells to recognize both *Amb a I.3* peptides and homologous *Amb a I.1* peptides was determined. The peptides used in this study are listed below. The sequences for the *Amb a I.1* homologous *Amb a I.3* peptides were based on an alignment of the *Amb a I.3* sequence to the *Amb a I.1* sequence.

10

***Amb a I.1* peptides**

AMB 1-2.1 KGTVGGKDGDIYTVTSELDDD VAN  
AMB 1-4.1 AENRKALADCAQGFGKGTVGGK DGD  
AMB 2-1.1 GPAAPRAGSDGDAISISGSSQ  
AMB 3-1.1 GSYAIGGSASPTILSQGNRFCAP DERSK  
AMB 3-4.1 FFQVVNNNYDKWGSY AIGGSASPT  
AMB 4-3.1 VLENGAIFVASGVDPVLPEQSAGMIP

20

***Amb a I.3* peptides**

AMB 1-2.15 KGTYGGK WGDVYTVTSNL DDD VAN  
AMB 1-4.15 ENNRQALADCAQGF A KGTYGGK WGD  
AMB 2-1.15 GPPILRQASDGDTINVAGSSQ  
AMB 3-1.15 GTYAIGGSSAP TILCQGNRF LAPDDQIK  
AMB 3-4.15 FFQVVNNNYDRWGT Y AIGGSSAP T  
AMB 4-3.15 LLENGAIFVTSGSDPV LTPVQSAGMIP

PBMC from ragweed allergic patients were stimulated with 20 mg/ml recombinant 30 *Amb a I.1* protein as described in Example VI with the addition of cultures of PBMC which were stimulated with 20 mg/ml recombinant *Amb a I.3* protein. Assays were performed as described in Example VI except the homologous *Amb a I.3* peptides were also tested at various doses. In these experiments an individual S.I. of 2.0 or greater was used in calculating the mean S.I. Fig. 19 shows the results from a set of 9 matched patients 35 stimulated with either recombinant *Amb a I.1* protein or recombinant *Amb a I.3* protein and tested on the set of *Amb a I.1* peptides described above. Each bar represents the P.I. The dark bar represents cells stimulated with recombinant *Amb a I.1* protein and tested with *Amb a I.1* peptides, whereas, the stippled bar represents cells stimulated with recombinant *Amb a I.3* protein and tested with *Amb a I.1* peptides. With two exceptions, the results directly

parallel each other, indicating that cells stimulated with recombinant *Amb a I.3* proteins recognize *Amb a I.1* derived peptides comprising at least one T cell epitope. One exception is that cells primed with *Amb a I.3* protein recognize the RAE 7.D peptide poorly compared to recombinant *Amb a I.1* stimulated cells. In addition, cells stimulated with recombinant 5 *Amb a I.3* protein gave stronger responses overall to *Amb a I.1* derived peptides from Region 4 than the recombinant *Amb a I.1* stimulated cells, particularly peptide RAE 28.1-2.

Cells were also stimulated with either recombinant *Amb a I.1* or recombinant *Amb a I.3* and tested for reactivity with *Amb a I.3* derived peptides (Fig. 20). The results indicate 10 that cells stimulated with recombinant *Amb a I.1* protein recognize the *Amb a I.3* derived peptides in a similar pattern to that of cells stimulated with recombinant *Amb a I.3* protein. In Fig. 20 the bars represent the P.I. and the number above the bar the mean S.I. in the nine 15 patients. The ".15" designation following the peptide name indicates that the peptide is derived from the *Amb a I.3* protein. The data suggests a high degree of cross-reactivity at the T cell level between the *Amb a I.1* protein and the *Amb a I.3* protein.

15

#### Example X IgE Analysis With Peptides Derived From *Amb a I.1*

To analyze IgE reactivity of peptides derived from the *Amb a I.1* protein, a direct 20 binding ELISA was performed according to the procedure described in Example III. The source of IgE for this analysis was a pool of ragweed allergic patient plasma from 38 ragweed skin test positive patients. The ELISA protocol was the same as Example III except 25 that the antigen coating with peptides and proteins was a concentration of 10 mg/ml at 100 mls/well. Fig. 21 shows the graphed results of this assay demonstrating strong reactivity to both SPE and biochemically purified *Amb a I.1*. By this assay there is no detectable binding to any of the peptides. A set of these assays was also run with rabbit and mouse antisera to demonstrate that the coating of the peptides onto these plates was successful.

25

#### Example XI Further T Cell Epitope Fine Map Studies With *Amb a I.1*

To further define the regions of *Amb a I.1* specific T cell reactivity, selected peptides 30 from Figure 14 were analyzed. *Amb a I.1* specific T cells lines were derived from 28 ragweed allergic patients as described in Example VI. These lines were assessed for their 35 ability to proliferate in response to the peptide in the presence of autologous EBV transformed antigen presenting cells by the uptake of tritiated thymidine as described previously. Several lysine substituted peptides derived from the peptide RAE 70.1 sequence were designed to increase the solubility of the peptide from Region 2. These peptides shown in Figure 22 were tested against non-substituted controls to determine if the modification resulted in a change in T cell reactivity. In addition, truncations of peptide AMB 4-6.1 (amino acid residues 347-377) were tested to further refine the relative T cell reactivity in that region.

Figure 22 shows the responses of the 28 T cell lines to these peptides as analyzed by the positivity index, the mean stimulation index and the percent of positive responses. The positivity index, as defined previously, is the mean stimulation index multiplied by the percent of patients responding to a peptide. Responses to peptides were considered positive if they were greater or equal to 2 fold over background. Figure 22 demonstrates that certain lysine substituted peptides in the 182-216 sequence resulted in greater T cell responses, indicating that not all substitutions are recognized equally by a given T cell line. In addition, the T cell responses to the lysine modified peptides having a substitution of cysteine at position 212 with serine reflect the responses of T cell lines tested with peptides without the lysine substitutions. Thus, the decrease in response to AMB 2-10.1, with a substitution of leucine for cysteine at 212, reflects the decrease in the percentage of patients responding to this peptide relative to peptides AMB 2-9.1 and AMB 2-11.1 which contain serine and glutamic acid substitutions, respectively, at position 212.

In addition, the truncation peptides derived from peptide AMB 4-6.1 showed indistinguishable responses based on this analysis. Thus, the shortest sequence, 345-370 was chosen for further analysis. Substitutions at positions 360 and 361 were made to increase the stability of the AMB 4-9.1 peptide which contained an acid-sensitive aspartic acid-proline bond. As shown in Figure 23, three peptides were synthesized in which the proline at position 361 was substituted with either alanine, serine or glycine (AMB 4-9.1DA (SEQ ID NO:147), AMB 4-9.1DS (SEQ ID NO:148), and AMB 4-9.1DG (SEQ ID NO:149), respectively). Similarly, five peptides were synthesized in which the aspartic acid at position 360 was substituted with the following: glutamic acid (AMB 4-9.1EP, SEQ ID NO:142); asparagine (AMB 4-9.1NP, SEQ ID NO:143); alanine (AMB 4-9.1AP, SEQ ID NO:144); serine (AMB 4-9.1SP, SEQ ID NO:145); and glutamine (AMB 4-9.1QP, SEQ ID NO:146). This group of modified peptides were tested to determine whether a change in T cell reactivity occurred. 28 *Amb a I.1* specific T cell lines were analyzed by the relative strength of the response to the modified peptides in a given T cell line compared to the overall response to all peptides from that line. Figure 23 shows the ranked sum of the strongest three peptide responses in the 28 T cell lines. The ranked sum was determined as described previously with the strongest response to a peptide given a value of 3, the second strongest a 2, and the third strongest a value of 1. The values from all 28 lines were then added to obtain the ranked sum. Figure 23 indicates that the modified peptide AMB 4-9.1DA elicits T cell responses similar to those of the native sequence AMB 4-9.1. In contrast, other substitutions, while eliciting strong responses do not rank as highly in this analysis.

Based in part on the above described analyses, five peptides were selected for further study (AMB 1-2.1, AMB 2-6.1, AMB 2-9.1, AMB 3-5.1 and AMB 4-9.1). An additional peptide, RA-02.1 (SEQ ID NO:150) was also synthesized and tested to confirm that the serine substitution at position 212 was recognized by *Amb a I.1* specific T cell lines. The T cell reactivity of these peptides in 32 different *Amb a I.1* specific T cell lines was determined

as described previously. Figure 24 demonstrates the relative positivity indices, percent of positive responses and mean stimulation indices of these six peptides compared to the overall response to *Amb a* 1.1. This figure shows that peptide AMB 4-9.1 elicits the strongest response in a high percentage of patients.

5 In contrast to previous data, the response to the 182-216 peptide (AMB 2-6.1) and substituted variants within this sequence (RA-02.1) gave weaker stimulation indices. This could reflect a potential toxic effect of the peptides in the T cell assays. To investigate this possibility, an assay was conducted to determine the ability of the peptides to inhibit T cell proliferation. Inhibition was defined as proliferation of a T cell line in response to the 10 peptide which was less than half the proliferation of media control T cell lines plus autologous EBV transformed antigen presenting cells. As seen in Table V, the percent of patients in which inhibition of T cell proliferation is seen is far greater in cultures where the AMB 2-6.1, RA-02.1, and AMB 2-9.1 peptides are present as compared to AMB 1-2.1 or AMB 4-9.1. This data suggests that AMB 2-6.1, RA-02.1 and AMB 2-9.1 may be inhibitory 15 due to toxicity in *in vitro* T cell line culture. This is further supported by the finding that these peptides also inhibit a T cell line from patient 956.2 which is specific for an irrelevant antigen, native *Feld* I, as shown in Figure 26.

TABLE V

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ASSESSMENT OF PEPTIDE TOXICITY IN *Amb a* 1.1  
PRIMED SECONDARY T CELL CULTURE

	peptide	#/(%)patients	low dose <u>only</u>	high dose <u>only</u>	both
25	AMB 1-2.1	3/46 (7)	2/3	1/3	0
30	AMB 2-6.1	14/44 (32)	0	11/14	3/14
	RA-02.1	19/45 (41)	0	15/19	4/19
	AMB 2-9.1	23/38 (61)	0	14/23	9/23
	AMB 4-9.1	1/46 (2)	0	0	1/1
35	AMB 3-5.1	19/46(41)	0	17/19	2/19

Legend: Toxicity defined as proliferation, as assessed by incorporation of tritiated thymidine, which is less than half the proliferation of media control of an *Amb a* 1.1

stimulated T cell line plus APC. Low dose = 5 mg/ml peptide in secondary assay, high dose = 50 mg/ml peptide.

5 A peptide from Region 2, RAE 70.1 (SEQ ID NO:44) was modified to minimize dimerization via disulfide linkages by substituting serine for cysteine at position 212. In addition, peptide RAE 70.1 was divided into two fragments and modified to increase solubility and to further define the residues necessary for T cell reactivity. As shown in Figure 25, a fragment of RAE 70.1 (amino acid residues 194-216) was modified by substitution or addition of amino acids which would increase the hydrophilicity and decrease 10 the pI of the peptide to thereby increase solubility.

15 Peptides Amb 2-18.1 (SEQ ID NO:126), Amb2-19.1 (SEQ ID NO:127), Amb2-20.1 (SEQ ID NO:128) and Amb2-21.1 (SEQ ID NO:129) correspond to different lengths of the original peptide which were synthesized (including the serine substitution at position 212) and analyzed to determine solubility. It was found that peptide Amb2-19.1 (residues 200- 217) was most soluble. Thus, substitutions and/or amino acid additions to this peptide were made in order to further increase the solubility while maintaining T cell reactivity. For example, in peptides Amb2-22.1 (SEQ ID NO:130) and Amb2-23.1 (SEQ ID NO:131) isoleucine at position 201 was substituted with glutamic acid (Amb2-22.1) or lysine (Amb2- 23.1) to lower the pI of the resulting peptide and avoid precipitation of the peptide at 20 physiological pH. Similarly, the following peptides were also synthesized with various substitutions or additions designed to decrease the pI and increase the hydrophilicity of the peptide, each is shown in Figure 25: Amb2-26.1 (SEQ ID NO:132); Amb2-28.1 (SEQ ID NO:133); Amb2-30.1 (SEQ ID NO:134); Amb2-32.1 (SEQ ID NO:135); Amb2-33.1 (SEQ ID NO:136); Amb2-34.1 (SEQ ID NO:137); Amb2-35.1 (SEQ ID NO:138); Amb2-36.1 25 (SEQ ID NO:139); Amb2-37.1 (SEQ ID NO:140); and Amb2-38.1 (SEQ ID NO:141).

30 Figures 27 and 28 show representative examples of proliferation of two individual *Amb a I.1* specific T cell lines to peptides selected from those shown in Figure 25. Assays were performed as described previously. Both Figure 27 and Figure 28 indicate a hierarchy of responses to these peptides. Patient 119 shown in Figure 27, has a hierarchy of response from strongest to weakest as follows: AMB2-23.1>AMB2-22.1>AMB2-30.1>AMB2- 26.1>AMB2-33.1>AMB2-32.1>AMB2-18.1>AMB2-19.1. Peptides AMB2-34.1, AMB2- 35.1, and AMB 2-9.1 did not elicit significant T cell proliferation in this patient. Patient 35 1199 shown in Figure 28 demonstrated strong responses to AMB 2-26.1>AMB 2-22.1>AMB 2-33.1. T cells from this patient showed less than 2 fold over background responses to all other peptides. These responses are not significantly different from the media control of the T cell line plus autologous EBV antigen presenting cells.

To more closely examine the T cell epitopes within the above set of peptides to which *Amb a I.1* specific T cell lines respond, a T cell clone was generated by limiting dilution from an *Amb a I.1* specific T cell line stimulated with AMB 2-10.1. Briefly, an *Amb a I.1* specific

T cell line from patient 776 was shown to respond to AMB 2-10.1 in a proliferation assay as described previously. To generate a AMB 2-10.1 specific T cell clone, *Amb a* 1.1 specific T cells were plated at 0.3 cells/well in a V-bottom 96 well plate with 20,000 irradiated autologous EBV transformed antigen presenting cells, AMB 2-10.1 at 40 mg/ml, 5 leukoagglutin at 1 mg/ml, and recombinant human IL-2 and IL-4 at 10 units/ml in a total volume of 100ml/well. Plating the T cells at 0.3 cells/well insures not more than 1 T cell/well can potentially proliferate to the peptide with the progeny of that T cell representing a clonal population. After five days, wells received an additional 15 units/ml of recombinant 10 human IL-2 and IL-4 to expand peptide-specific T cells. This addition of IL-2 and IL-4 was repeated again every three days for the duration of the culture period. Twelve days after the initiation of culture, T cells in the wells were restimulated with 20,000 irradiated EBV 15 antigen presenting cells and 40 mg/ml AMB 2-10.1. This was repeated again 20 days after the initial stimulation. T cells from wells which showed signs of growth were separated from cell debris by density centrifugation as described previously in the generation of T cell lines. The T cell clone was then expanded with additional IL-2 and IL-4 at 10 units/ml until there were significant numbers to assay for proliferation.

Assessment for T cell proliferation to selected peptides from those shown in Figure 25 was performed as described previously for T cell lines. The results of a representative assay using this AMB 2-10.1 specific T cell clone are shown in Figure 29. The data indicates 20 that the T cell epitope recognized by this clone is contained by all the peptides except AMB 2-34.1 and AMB 2-35.1. Thus, a substitution of the tryptophan at position 208 eliminates the ability of the T cell clone to respond to these peptides. This data suggests that substitutions on the N and C terminal ends of the truncated peptides does not effect the recognition of the peptides by the T cell clone and, thus, does not alter the residues which comprise the T cell 25 epitope.

#### Example XII    Histamine Release Analysis With Purified *Amb a* 1.1 and With Peptides Derived From *Amb a* 1.1

The objective of the histamine release analysis was to compare the effects of *Amb a* 30 1.1 or *Amb a* 1.1-derived peptides in an *in vitro* allergic response system. The release of histamine via IgE recognition and IgE receptor crosslinking on viable cells directly assays the allergic potential of a protein antigen.

The histamine release assay used for these studies is based on the detection of an 35 acylated derivative of histamine using a specific monoclonal antibody (Morel, A.M. and Delaage, M.A. (1988) *J. Allergy Clin. Immunol.* 82:646-654). The assay was performed in two steps: 1) the release of histamine from basophils present in heparinized whole blood in the presence of different concentrations of protein or peptide; and 2) the assay of histamine present in the supernatants of the release reactions following cell removal by centrifugation.

The reagents for this second step are available commercially as a competitive radioimmunoassay from AMAC Inc. (Westbrook, ME).

Heparinized whole blood was drawn from ragweed allergic patients. The test protein Amb a I.1 and peptides AMB 1-2.1, AMB 2-9.1, AMB 4-9.1, and AMB 3-5.1 were each 5 diluted to 2X the final release concentration in PACM buffer (PIPES 25mM, NaCl 110mM, KCl 5.0 mM, human serum albumin 0.003% (w/v), CaCl<sub>2</sub> 5mM, MgCl<sub>2</sub> 2mM, pH7.3) and 0.2 ml of each dilution was added to a 1.5 ml polypropylene tube. A 0.2ml aliquot of blood was then added to each tube and the release reactions started by inversion of the tubes. A 10 negative control of 0.2 ml blood incubated with 0.2 ml PACM buffer was also included. The release reactions were performed for 30 minutes at 37°C. The tubes were then centrifuged in a microfuge at 1500 RPM for 3 minutes and the supernatants were carefully removed and 15 analyzed or stored at -20°C for later analysis. To analyze the total histamine released, 0.1 ml blood was diluted with 0.9 ml PACM buffer and boiled 3 minutes. This tube was then centrifuged 2 minutes at 12,000 RPM and the supernatant was removed and save for analysis.

15 For the competition radioimmunoassay, a 50 ml aliquot of each release supernatant was mixed with 150 ml of histamine release buffer (supplied with the kit from AMAC). The diluted supernatant (100 ml) was added to a kit tube containing an acylation reagent. A 50 ml aliquot of acylation buffer was then immediately added and the tube mixed by vortexing. The acylation reactions were incubated at least 30 minutes at room temperature. A set of 20 histamine standards supplied with the kit were acylated at the same time. 50 ml of each acylation reaction was then placed in a tube coated with a monoclonal antibody which 25 specifically recognizes the acylated form of histamine. A 0.5 ml aliquot of <sup>125</sup>I-labelled tracer was added and the tubes were incubated at least 18 hours at 4°C. The solutions were aspirated from the tubes and the radioactivity bound to the tube was measured on a gamma counter (Cobra 5005, Beckman, Inc.) for two minutes per tube.

A standard curve was generated from the histamine standard counts and graphed on a semi-log plot. Since this is a competitive assay (the <sup>125</sup>I-labelled tracer competes with acylated histamine in the samples for binding to the antibody-coated tubes), the lower the 30 number of radioactive counts measured, the greater the amount of histamine in the sample. The amount of histamine in each sample data point was extrapolated from the standard curve using a computer statistical program (StatView for the MacIntosh). The assay is sensitive to 0.2 nM histamine.

To study whether peptides derived from the *Amb a I.1* sequence could induce 35 histamine release, blood samples from 8 ragweed-allergic patients who exhibited histamine release to native *Amb a I.1* were analyzed with AMB 1-2.1, AMB 2-9.1, AMB 4-9.1 and AMB 3-5.1. Six or more 10-fold dilutions of *Amb a I.1* starting at 10 mg/ml (approximately 0.66 mM) were analyzed. Five five-fold dilutions of each peptide were also analyzed (ranging from 50 mg/ml to 0.08 mg/ml, this is approximately 15 mM to 24 nM in concentration for each peptide). The peptide concentrations were selected to encompass the

higher end of the concentration curve. Figure 30 shows the representative results from 1 of the patients (#1273). The graph shows the concentration of each antigen in mg/ml versus the percent of total histamine released. The results are presented as the percent of the total histamine released for that patient, since patients vary greatly in their overall releasable histamine levels. This variability is likely to result from variation in the number of basophils per ml between patients and the histamine content of each basophil. As Figure 30 shows, 5 *Amb a I.1*, at all the concentrations tested, yields a high level of histamine release in this patient. Similar experiments using blood samples from other patients have shown that for some patients the *Amb a I.1* concentration does not become limiting until  $10^{-5}$ - $10^{-6}$  mg/ml. 10 By contrast, there is no discernible histamine release to any of the four *Amb a I.1*-derived peptides which were used. Similar results were obtained for seven other patients similarly tested.

In another experiment, plasma samples from 94 ragweed-allergic individuals, who 15 tested positive for IgE binding to *Amb a I.1* were analyzed for IgE binding to peptides *Amb 1-2.1*, *Amb 2-36.1* and *Amb 4-9.1* by direct binding ELISA as described above. Plasma samples were studied at a 1:3 and 1:30 dilution. None of the 94 patients tested had measurable IgE binding to either *Amb 1-2.1* or *Amb 2-36.1* (data not shown). Six patients had a low level of IgE binding to *Amb 4-9.1* (data not shown). These results indicate that 20 approximately 7% of ragweed-allergic patients have low plasma IgE levels to *Amb 4-9.1*.

In order to assess the relative contribution of *Amb 4-9.1*-specific IgE to the overall 25 level of IgE to *Amb a I.1*, plasma from one patient with IgE to *Amb 4-9.1* was depleted of IgE to *Amb 4-9.1* by repeatedly absorbing the plasma on plates coated with *Amb 4-9.1-03* conjugated ovalbumin. A mock absorption was performed in parallel using plates coated with *Amb 1-2.1* conjugated to ovalbumin. Previous experiments have shown that a somewhat higher O.D. signal can be obtained when the ovalbumin conjugate is used for plate 30 coating. After absorption, the original, non-absorbed plasma samples were analyzed for IgE binding to *Amb a I.1* in a direct assay. The efficiency of absorption was verified by also analyzing binding to *Amb 4-9.1*. The results indicated that absorption with *Amb 4-9.1* efficiently removed any detectable binding to *Amb 4-9.1*. The mock absorption with *1-2.1* yielded a slight decrease in the O.D. signals seen in the unabsorbed sample, as indicated by 35 the shift curve for *Amb 1-2.1* absorbed plasma to the left of the curve for unabsorbed plasma. The curve for *Amb 4-9.1* shifted very slightly to the left of the *Amb 1-2.1*-absorbed plasma curve. This result suggests that IgE binding to *Amb 4-9.1* represents a minor but measurable component of the overall level of IgE to *Amb a I.1*.

To further assess the relative contribution of anti-*AMB 4-9.1-03* IgE to the overall 35 anti-*Amb a I.1* response, a competition ELISA was performed using *Amb 4-9.1* and *Amb a I.1* in solution to compete for binding to *Amb a I.1*. For a negative control, competition was also performed using *Amb 1-2.1*. This experiment shows that *Amb 4-9.1* does not detectably compete for IgE binding to *Amb a I.1* at concentrations where very effective competition is

seen with soluble *Amb a* I.1. The results (data not shown) indicate that the change in IgE binding detected to Amb 4-9.1 when the plasma IgE response is competed with *Amb a* I.1 and Amb 4-9.1. As expected, incubation of the plasma sample with soluble Amb 4-9.1 can compete for binding to Amb 4-9.1. However, soluble *Amb a* I.1 competes much more 5 efficiently since much lower concentrations (in  $\mu$ /mL) of *Amb a* I.1 protein than Amb 4-9.1 peptide are required to compete for binding to Amb 4-9.1. This difference is even more distinct if the comparison is made on an equimolar basis since the peptide is in greater than ten-fold excess relative to *Amb a* I.1 at equivalent  $\mu$ g/mL concentrations. This result 10 suggests that in this patient sample, the portion of the IgE response detected which is directed to an epitope defined by the Amb 4-9.1 peptide sequence has lower affinity for Amb 4-9.1 than for *Amb a* I.1.

All peptide candidates were also examined for their ability to release histamine *in vitro* from basophils of ragweed-allergic patients. The objective of the histamine release analysis was to measure the effects of *Amb a* I.1 peptides Amb 1-2.1, Amb 2-36.1 and Amb 15 4-9.1 *in vitro* allergic response system. Heparinized whole blood from ragweed-allergic patients was incubated with different concentrations of each peptide (at 0.08-50  $\mu$ g/mL) or *Amb a* I.1 (at  $10^{-7}$  to 10  $\mu$ g/mL). The levels of histamine release were measured using commercially available radioimmunoassay (Amac, Inc., Westbrook, ME). No patient with histamine release to native *Amb a* I.1 (n=19), released histamine to peptides Amb 1-2.1 20 (n=19), Amb 2-36.1 (n=19) and Amb 4-9.1 (n=14) (data not shown). Histamine release to Amb 4-9.1 was also not detected when blood from two patients with anti Amb 4-9.1 antibodies was analyzed (data not shown). The failure of Amb 4-9.1 to induce histamine release in two patients with IgE to Amb 4-9.1 is possibly due to the inability of the peptide to mediate the crosslinking of the IgE receptors on blood basophils.

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### Example XIII T Cell Responses to Ragweed Peptides

Peripheral blood mononuclear cells (PBMC) were purified by lymphocyte separation medium (LSM) centrifugation of 60 ml of heparinized blood from ragweed-allergic patients who exhibited clinical symptoms of seasonal rhinitis and were skin prick test positive for 30 ragweed. T cell lines were established from these cells by stimulation of 1-2  $\times 10^6$  PBMC/ml in RPMI-1640 containing 5% human AB serum (complete medium) with native *Amb a* I.1 at 20 mg/ml for 5-6 days at 37°C in a humidified CO<sub>2</sub> incubator. Viable cells were purified by LSM centrifugation and cultured in complete medium supplemented with 5 units recombinant human IL-2/ml and 5 units recombinant human IL-4/ml for up to three 35 weeks until the cells no longer responded to lymphokines and were considered "rested". The ability of the T cells to proliferate to *Amb a* I.1 sequence-derived synthetic peptides was then assessed.

For assay, 2  $\times 10^4$  rested cells were restimulated in the presence of 2  $\times 10^4$  autologous Epstein-Barr virus (EBV)-transformed B cells (gamma-irradiated with 25,000

RADS) or  $5 \times 10^4$  autologous PBMC (3,500 RADS) with various concentrations of *Amb a* I.1 synthetic peptides in a volume of 200 ml complete medium in duplicate or triplicate wells in 96-well round bottom plates for 3 days. Each well then received 1 mCi tritiated thymidine for 16-20 hours. The counts incorporated were collected onto glass fiber filter mats and 5 processed for liquid scintillation counting. Table III shows the results of a representative assay. The maximum response in a titration of each peptide is expressed as the S.I. or stimulation index. The S.I. is the CPM incorporated by cells in response to peptide divided by the CPM incorporated by cells in medium only. An S.I. value greater than the background level is considered "positive" and indicates that the peptide contains a T cell epitope.

10 However, only individual S.I. values above 2.0 (a response two-fold or greater over background) were used in calculating mean stimulation indices for each peptide for the group of patients tested. The results shown in Table VI demonstrate that this patient (#1715) responds very well to peptides RAE 74.1, Amb 4-9.1, RAE 61.1, RAE 62.1. This indicates 15 that these peptides contain *Amb a* I.1 T cell epitopes recognized by T cells from this particular allergic patient.

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TABLE VI

<u>Antigen</u>	<u>Concentration (mg/ml)</u>	<u>CPM</u>	<u>S.I.</u>
5    Medium	--	452	1
Amb a I.1	10	47822	
	100	63494	139.9
10    RAE 61.1	5	767	
	50	5910	13.0
RAE 80.1	5	640	
	50	1067	2.4
15    RAE 45.1	5	589	
	50	1106	2.4
20    RAE 75.1	5	1094	2.4
	50	521	
RAE 62.1	5	2017	
	50	4923	10.8
25    RAE 65.1	5	1048	
	50	462	
30    RW 31	5	892	
	50	1309	2.9
RAE 74.1	5	18992	
	50	10633	7.8
35    AMB 4-9.1	5	14065	
	50	8018	31.0

The above procedure was followed with a number of other patients. Individual patient results were used in calculating the mean S.I. for each peptide if the patient responded to the *Amb a 1.1* protein at an S.I. of 2.0 or greater and the patient responded to at least one peptide derived from *Amb a 1.1* at an S.I. of 2.0 or greater. A summary of positive 5 experiments from 57 patients is shown in Fig. 31. The bar represents the cumulative rank of the peptide response in the 57 patients. To determine the cumulative rank, the 5 peptides with the highest S.I. in each patient were determined and assigned a numerical rank in descending order with 5 representing the strongest response. The ranks for each peptide were then summed in the 57 patients to determine the cumulative rank for the peptide. The 10 number above each bar in parenthesis is the percentage of the positive responses (S.I. of 2.0 or greater) from the group of patients to that peptide. In parentheses above each bar is the mean S.I. of the positive responses. The cumulative rank sum represents both the strength of the response (S.I.) and the frequency of a response to a peptide in the group of patients tested. For example, peptide AMB 4-9.1 had the highest cumulative rank so it was the best peptide 15 response in the overall population of 57 even though it did not have the highest mean S.I. Similarly, RAE 27.1 had the highest mean S.I. but not the best cumulative rank. Thus, the response to RAE 27.1 was strong when it occurred but it did not occur as frequently in the population as the response to other peptides. Based on the data as shown in Fig. 31, peptides AMB 4-9.1, RAE 74.1, AMB 3-1.1, AMB 2-36.1, RAE62.1 and AMB 1-2.1 were identified 20 as the peptides with the strongest T cell reactivity

#### Example XIV Selection of Peptides For Use in Human Therapy

To determine whether a peptide (candidate peptide) or a combination of candidate peptides are likely to comprise a sufficient percentage of T cell epitopes of ragweed pollen 25 protein to induce T cell non responsiveness in a substantial percentage of a population of individuals sensitive to ragweed pollen protein and therefore, be a suitable composition for human therapy, the following evaluation is used. Candidate peptides are selected based, at least in part on the mean human T cell stimulation index for each of the candidate peptides in the set of peptides tested and the positivity index (the mean S.I. multiplied by the percent of 30 positive responses) of the candidate peptides in the set of peptides tested. For example, the results in Example XIII (Fig. 31) indicate a number of peptides which contain strong T cell reactivity, and three of such peptides, Amb 1-2.1, Amb 2-36.1 and Amb 4-9.1, were selected as candidate peptides for use in human therapy. The human T cell stimulation index for each peptide tested including the stimulation index for the candidate peptides is summed. For 35 each individual, the human T cell stimulation index for the candidate peptides are divided by the sum of the human T cell stimulation indices of the remaining peptides in the set of peptides tested to determine a percent of T cell reactivity as shown below:

% of T cell Reactivity of candidate peptides =

$$\frac{\text{Sum of S.I.s for Each Candidate Peptide}}{\text{Sum of S.I.s of the set of overlapping peptides}} \times 100$$

5 A more liberal estimate of reactivity elicited by the candidate peptide, accounting for the overlaps giving rise to the total can be calculated as follows:

% of T cell Reactivity of Candidate Peptides =

$$\frac{N_t \text{ flanking peptide S.I.} + \text{Candidate S.I.} + C_t \text{ flanking peptide S.I.}}{\text{Sum of S.I.s from overlapping peptides}} \times 100$$

In this formula, "N<sub>t</sub> flanking peptide" refers to a peptide which comprises amino acid residues which overlap with amino acid residues located at the N-terminus of the candidate peptide in the amino acid sequence of the protein antigen from which the peptide is derived; "C<sub>t</sub> flanking peptide" refers to a peptide which comprises amino acid residues which overlap with amino acid residues located at the C-terminus of the candidate peptide in the amino acid sequence of the protein antigen from which the peptide is derived. In this calculation, stimulation indices for the candidate peptide, the N-terminal flanking peptide and the C-terminal flanking peptide are added and divided by the sum total of the stimulation indices for the entire set of overlapping peptides to obtain a percent of T cell reactivity for the candidate peptides. If a combination of two or more candidate peptides is selected each of which contains amino acid residues which overlap, this calculation cannot be used to determine a percent of T cell reactivity for each candidate separately. However this is not the case for selected candidates, Amb 1-2.1, Amb 2-36.1 and Amb 4-9.1.

25 The values obtained for the percentage of T cell reactivity for the candidate peptides in each individual tested can be expressed as a range of lower and higher values of the results of the above described calculations. The mean percentage of T cell reactivity elicited by the candidate peptides can then be determined by averaging the values obtained for the individual responses to the candidate peptides.

30 For candidate peptides Amb 1-2.1, Amb 2-36.1 and Amb 4-9.1, secondary *Amb a 1.1* T cell cultures derived from 57 ragweed-allergic subjects were analyzed for reactivity to an overlapping set of Amb a 1.1 peptides, and the highest stimulation index greater than or equal to 2.0 in response to each peptide was recorded for each individual tested. The data were then analyzed by the equation above for each individual tested, and the mean percentage of T cell reactivity was determined by averaging all the values obtained for the individual responses to the candidate peptides, Amb 1-2.1, Amb 2-36.1 and Amb 4-9.1, with the following results.

Candidate Peptides	Range of mean percentage of T cell reactivity	Frequency of response to at least one peptide
Amb 1-2.1 + Amb 2-36.1 +	26-35%	91%
Amb 4-9.1		

These values meet the criteria for a combination of candidate peptides described earlier.

5    Example XV Administration of Peptides to Humans for Treatment of Allergy to Ragweed  
A. Composition of Phase I Formulation

For Phase I clinical trials, the drug product was a multipeptide formulation comprising three peptides, Amb 1-2.1, Amb 2-36.1 and Amb 4-9.1, which together were determined to cover a sufficient percentage of T cell epitopes of a population of individuals 10 as discussed in Example XIV. Each peptide was purified to homogeneity (at least 97% pure) by known methods and a multipeptide formulation was prepared in accordance with procedures discussed earlier. The multipeptide formulation used in this Phase I clinical Study was in the form of a freeze-dried powder cake comprising 1500 µg/L (when reconstituted) of each of the peptides, Amb 1-2.1, Amb 2-36.1 and Amb 4-9.1, in a single 15 vial. The excipients used were 5% mannitol and 0.05 M sodium phosphate buffer system in a single vial. The formulation was reconstituted just prior to use with sterile water for injection resulting in a solution with a pH of ca. 7.5. Normal saline (0.9%) was used for any dilutions beyond initial reconstitution.

20    B. Human Phase I Clinical Studies

This study was a double blind placebo-controlled safety study of thirty-six patients conducted at Johns Hopkins Asthma and Allergy Center Baltimore. Twenty-four patients received active therapy while twelve received placebo.

After selection by demonstration skin test sensitivity to ragweed extract, the patients 25 were tested for allergic sensitivity to study medication (active or placebo) prior to the first treatment by intracutaneous tests with increasing concentrations of the therapy. first Cohort (Cohort A) of sixteen patients received increasing weekly doses of 7.5, 75, 250, 750, and 1500 µg (per peptide) of the multipeptide formulation and four received placebo for five weeks. Cohort B of ten patients (6 active, 4 placebo) initiated a five week course of weekly 30 fixed dose therapy (250 µg per peptide or placebo). After all patients in Cohort A completed the fourth week of therapy, a third Cohort (Cohort C) of ten patients (6 active, 4 placebo) began a five-week course of weekly fixed dose therapy (750 µg per peptide or placebo). All patients underwent skin testing to ragweed extract and study medication (active or placebo) three weeks post treatment. Physical evaluation and clinical laboratory safety studies were 35 done at baseline and at eight weeks.

Preliminary data indicates that all doses were tolerated and the multipeptide formulation appears to be safe. Although this study was designed to evaluate safety, preliminary data suggests that the multipeptide formulation may possibly be reducing late phase responses to ragweed allergen in the skin. Further statistical analysis of the Phase I

5 Clinical studies are pending. Phase II Clinical Studies are pending in the United States and Canada

### Equivalents:

10 Although the invention has been described with reference to its preferred embodiments, other embodiments can achieve the same results. Variations and modifications to the present invention will be obvious to those skilled in the art and it is intended to cover in the appended claims all such modifications and equivalents that follow in the true spirit and scope of this invention.

- 54 -

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

5

(i) APPLICANT: Kuo, Mei-chang  
Garman, Richard  
Greenstein, Julia  
Evans, Sean  
10 Amsberry, Kent  
Shaked, Ze'ev

10

(ii) TITLE OF INVENTION: T CELL EPITOPES OF THE MAJOR ALLERGENS  
FROM AMBROSIA ARTEMISIIFOLIA

15

(iii) NUMBER OF SEQUENCES: 150

## (iv) CORRESPONDENCE ADDRESS:

20

(A) ADDRESSEE: IMMULOGIC PHARMACEUTICAL CORPORATION  
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(C) CITY: Waltham  
(D) STATE: MA  
(E) COUNTRY: USA  
(F) ZIP: 02154

25

## (v) COMPUTER READABLE FORM:

30

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: ASCII Text

35

## (vi) CURRENT APPLICATION DATA:

40

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

## (vi) PRIOR APPLICATION DATA:

45

(A) APPLICATION NUMBER: US08/298,542  
(B) FILING DATE: August 30, 1994

50

## (viii) ATTORNEY/AGENT INFORMATION:

45

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(B) REGISTRATION NUMBER: 35,729  
(C) REFERENCE/DOCKET NUMBER: 001.4 US

55

## (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 466-6000  
(B) TELEFAX: (617) 466-6040

50

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

55

(A) LENGTH: 1223 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

5 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..1188

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

10	ATG GGG ATC AAA CAC TGT TGT TAC ATC TTG TAT TTT ACC TTA GCC CTT Met Gly Ile Lys His Cys Cys Tyr Ile Leu Tyr Phe Thr Leu Ala Leu	48
	1 5 10 15	
15	GTC ACT TTG CTG CAA CCT GTT CGT TCT GCC GAA GAT CTC CAG GAA ATC Val Thr Leu Leu Gln Pro Val Arg Ser Ala Glu Asp Leu Gln Glu Ile	96
	20 25 30	
20	TTA CCA GTT AAC GAA ACA AGG AGG CTG ACA ACA AGT GGA GCA TAC AAC Leu Pro Val Asn Glu Thr Arg Arg Leu Thr Thr Ser Gly Ala Tyr Asn	144
	35 40 45	
25	ATT ATA GAC GGG TGC TGG AGG GGC AAA GCC GAT TGG GCG GAA AAC CGA Ile Ile Asp Gly Cys Trp Arg Gly Lys Ala Asp Trp Ala Glu Asn Arg	192
	50 55 60	
30	AAA GCG TTA GCC GAT TGT GCC CAA GGT TTT GGG AAG GGA ACA GTG GGC Lys Ala Leu Ala Asp Cys Ala Gln Gly Phe Gly Lys Gly Thr Val Gly	240
	65 70 75 80	
35	GGA AAA GAT GGT GAT ATA TAC ACG GTC ACC AGT GAG CTA GAT GAT GAT Gly Lys Asp Gly Asp Ile Tyr Thr Val Thr Ser Glu Leu Asp Asp Asp	288
	85 90 95	
40	GTT GCA AAT CCA AAA GAA GGC ACA CTC CGG TTT GGT GCC GCC CAA AAC Val Ala Asn Pro Lys Glu Gly Thr Leu Arg Phe Gly Ala Ala Gln Asn	336
	100 105 110	
45	AGG CCC TTG TGG ATC ATT TTT GAA AGA GAT ATG GTG ATT CGT TTG GAT Arg Pro Leu Trp Ile Ile Phe Glu Arg Asp Met Val Ile Arg Leu Asp	384
	115 120 125	
50	AAA GAG ATG GTG GTA AAC AGT GAC AAG ACC ATC GAT GGC CGA GGG GCG Lys Glu Met Val Val Asn Ser Asp Lys Thr Ile Asp Gly Arg Gly Ala	432
	130 135 140	
55	AAA GTT GAA ATC ATT AAC GCT GGT TTC ACC CTT AAT GGT GTC AAG AAT Lys Val Glu Ile Ile Asn Ala Gly Phe Thr Leu Asn Gly Val Lys Asn	480
	145 150 155 160	
60	GTA ATC ATT CAT AAC ATA AAT ATG CAT GAT GTT AAA GTG AAT CCA GGA Val Ile Ile His Asn Ile Asn Met His Asp Val Lys Val Asn Pro Gly	528
	165 170 175	
65	GGC CTG ATT AAG TCC AAC GAT GGT CCA GCA GCT CCA AGA GCT GGT AGT Gly Leu Ile Lys Ser Asn Asp Gly Pro Ala Ala Pro Arg Ala Gly Ser	576
	180 185 190	
70	GAT GGT GAT GCT ATA AGT ATT TCT GGT AGT TCA CAA ATA TGG ATC GAC Asp Gly Asp Ala Ile Ser Ile Ser Gly Ser Ser Gln Ile Trp Ile Asp	624

- 56 -

	195	200	205	
				672
	CAT TGT TCG CTC AGT AAG TCT GTT GAT GGG CTG GTA GAT GCC AAG CTC			
	His Cys Ser Leu Ser Lys Ser Val Asp Gly Leu Val Asp Ala Lys Leu			
5	210	215	220	
	GGC ACC ACA CGC TTA ACC GTT TCC AAC AGC TTA TTC ACC CAA CAC CAG			720
	Gly Thr Thr Arg Leu Thr Val Ser Asn Ser Leu Phe Thr Gln His Gln			
	225	230	235	240
10	TTT GTA CTA TTA TTC GGG GCT GGT GAC GAA AAT ATT GAA GAT AGA GGC			768
	Phe Val Leu Leu Phe Gly Ala Gly Asp Glu Asn Ile Glu Asp Arg Gly			
	245	250	255	
15	ATG CTA GCA ACG GTC GCT TTC AAC ACG TTC ACT GAT AAC GTT GAC CAA			816
	Met Leu Ala Thr Val Ala Phe Asn Thr Phe Thr Asp Asn Val Asp Gln			
	260	265	270	
20	AGA ATG CCT AGA TGT CGA CAT GGG TTT TTC CAA GTC GTT AAC AAC AAC			864
	Arg Met Pro Arg Cys Arg His Gly Phe Phe Gln Val Val Asn Asn Asn			
	275	280	285	
25	TAT GAT AAA TGG GGA TCG TAT GCC ATC GGT GGT AGC GCG TCC CCA ACC			912
	Tyr Asp Lys Trp Gly Ser Tyr Ala Ile Gly Gly Ser Ala Ser Pro Thr			
	290	295	300	
30	ATA CTC AGC CAA GGG AAC AGA TTC TGC GCC CCC GAT GAA CGC AGC AAG			960
	Ile Leu Ser Gln Gly Asn Arg Phe Cys Ala Pro Asp Glu Arg Ser Lys			
	305	310	315	320
35	AAA AAT GTC CTA GGA AGG CAT GGT GAA GCC GCC GCA GAG TCG ATG AAG			1008
	Lys Asn Val Leu Gly Arg His Gly Glu Ala Ala Ala Glu Ser Met Lys			
	325	330	335	
40	TGG AAC TGG AGA ACG AAT AAA GAC GTG CTT GAA AAT GGT GCT ATT TTT			1056
	Trp Asn Trp Arg Thr Asn Lys Asp Val Leu Glu Asn Gly Ala Ile Phe			
	340	345	350	
45	GTT GCA TCC GGG GTC GAT CCA GTG CTA ACC CCT GAG CAA AGC GCA GGG			1104
	Val Ala Ser Gly Val Asp Pro Val Leu Thr Pro Glu Gln Ser Ala Gly			
	355	360	365	
50	ATG ATT CCA GCC GAA CCA GGA GAG TCC GCT CTA AGC CTC ACT AGT AGT			1152
	Met Ile Pro Ala Glu Pro Gly Glu Ser Ala Leu Ser Leu Thr Ser Ser			
	370	375	380	
	GCT GGT GTA CTC TCA TGC CAA CCC GGA GCA CCT TGC TAAGCACCCG			1198
	Ala Gly Val Leu Ser Cys Gln Pro Gly Ala Pro Cys			
	385	390	395	
55	ACCAATTACT AAGCACTTAT AATGA			1223

(2) INFORMATION FOR SEQ ID NO:2:

55

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 396 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear



- 58 -

Tyr Asp Lys Trp Gly Ser Tyr Ala Ile Gly Gly Ser Ala Ser Pro Thr  
 290 295 300  
 Ile Leu Ser Gln Gly Asn Arg Phe Cys Ala Pro Asp Glu Arg Ser Lys  
 5 305 310 315 320  
 Lys Asn Val Leu Gly Arg His Gly Glu Ala Ala Ala Glu Ser Met Lys  
 325 330 335  
 10 Trp Asn Trp Arg Thr Asn Lys Asp Val Leu Glu Asn Gly Ala Ile Phe  
 340 345 350  
 Val Ala Ser Gly Val Asp Pro Val Leu Thr Pro Glu Gln Ser Ala Gly  
 355 360 365  
 15 Met Ile Pro Ala Glu Pro Gly Glu Ser Ala Leu Ser Leu Thr Ser Ser  
 370 375 380  
 Ala Gly Val Leu Ser Cys Gln Pro Gly Ala Pro Cys  
 20 385 390 395

## (2) INFORMATION FOR SEQ ID NO:3:

25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1349 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 30 (ii) MOLECULE TYPE: cDNA

35 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..1194

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:  
 ATG GGG ATC AAA CAC TGT TGT TAC ATC TTG TAT TTT ACC TTA GCC CTT  
 Met Gly Ile Lys His Cys Cys Tyr Ile Leu Tyr Phe Thr Leu Ala Leu  
 1 5 10 15 48  
 45 GTC ACT TTG CTG CAA CCT GTT CGT TCT GCA GAA GAT GTT GAA GAA TTC  
 Val Thr Leu Leu Gln Pro Val Arg Ser Ala Glu Asp Val Glu Glu Phe  
 20 25 30 96  
 50 TTA CCT TCA GCT AAC GAA ACA AGG AGG AGC CTG AAA GCA TGT GAA GCA  
 Leu Pro Ser Ala Asn Glu Thr Arg Arg Ser Leu Lys Ala Cys Glu Ala  
 35 40 45 144  
 55 CAC AAC ATT ATA GAC AAG TGC TGG AGG TGC AAA GCC GAT TGG GCG AAT  
 His Asn Ile Ile Asp Lys Cys Trp Arg Cys Lys Ala Asp Trp Ala Asn  
 50 55 60 192  
 AAC CGA CAA GCG TTA GCC GAT TGT GCC CAA GGT TTT GCA AAG GGA ACC  
 Asn Arg Gln Ala Leu Ala Asp Cys Ala Gln Gly Phe Ala Lys Gly Thr  
 65 70 75 80 240

	TAC GGT GGA AAA CAT GGT GAT GTC TAC ACG GTC ACC AGT GAT AAA GAT Tyr Gly Gly Lys His Gly Asp Val Tyr Thr Val Thr Ser Asp Lys Asp 85 90 95	288
5	GAT GAT GTT GCA AAT CCA AAA GAA GGC ACA CTC CGG TTT GCT GCT GCC Asp Asp Val Ala Asn Pro Lys Glu Gly Thr Leu Arg Phe Ala Ala Ala 100 105 110	336
10	CAA AAC AGG CCC TTG TGG ATC ATT TTT AAA AGA AAT ATG GTG ATT CAT Gln Asn Arg Pro Leu Trp Ile Ile Phe Lys Arg Asn Met Val Ile His 115 120 125	384
15	TTG AAT CAA GAG CTT GTC GTA AAC AGC GAC AAG ACC ATC GAT GGC CGA Leu Asn Gln Glu Leu Val Val Asn Ser Asp Lys Thr Ile Asp Gly Arg 130 135 140	432
20	GGG GTG AAA GTT AAC ATC GTT AAC GCC GGT CTC ACC CTC ATG AAT GTC Gly Val Lys Val Asn Ile Val Asn Ala Gly Leu Thr Leu Met Asn Val 145 150 155 160	480
25	AAG AAT ATA ATC ATT CAT AAC ATA AAT ATC CAT GAT ATT AAA GTT TGT Lys Asn Ile Ile His Asn Ile His Asp Ile Lys Val Cys 165 170 175	528
	CCA GGA GGC ATG ATT AAG TCC AAC GAT GGT CCA CCA ATT TTA AGA CAA Pro Gly Gly Met Ile Lys Ser Asn Asp Gly Pro Pro Ile Leu Arg Gln 180 185 190	576
30	CAA AGT GAT GGT GAT GCT ATA AAT GTT GCT GGT AGT TCA CAA ATA TGG Gln Ser Asp Gly Asp Ala Ile Asn Val Ala Gly Ser Ser Gln Ile Trp 195 200 205	624
35	ATC GAC CAT TGC TCG CTC AGT AAG GCT TCC GAT GGG CTG CTC GAT ATC Ile Asp His Cys Ser Leu Ser Lys Ala Ser Asp Gly Leu Leu Asp Ile 210 215 220	672
40	ACC CTC GGC AGC TCA CAC GTG ACC GTT TCC AAC TGC AAA TTC ACC CAA Thr Leu Gly Ser Ser His Val Thr Val Ser Asn Cys Lys Phe Thr Gln 225 230 235 240	720
	CAC CAA TTT GTA TTA TTG CTC GGG GCT GAT GAC ACC CAT TAT CAA GAT His Gln Phe Val Leu Leu Gly Ala Asp Asp Thr His Tyr Gln Asp 245 250 255	768
45	AAA GGC ATG CTA GCA ACG GTA GCA TTC AAC ATG TTC ACC GAT CAC GTT Lys Gly Met Leu Ala Thr Val Ala Phe Asn Met Phe Thr Asp His Val 260 265 270	816
50	GAC CAA AGA ATG CCT AGA TGT AGA TTT GGG TTT TTC CAA GTC GTT AAC Asp Gln Arg Met Pro Arg Cys Arg Phe Gly Phe Phe Gln Val Val Asn 275 280 285	864
55	AAC AAC TAC GAC AGA TGG GGA ACG TAC GCC ATC GGT GGT AGC TCG GCC Asn Asn Tyr Asp Arg Trp Gly Thr Tyr Ala Ile Gly Gly Ser Ser Ala 290 295 300	912
	CCA ACT ATA CTC AGC CAA GGG AAC AGA TTC TTC GCC CCC GAT GAT ATC Pro Thr Ile Leu Ser Gln Gly Asn Arg Phe Phe Ala Pro Asp Asp Ile	960

- 60 -

305	310	315	320		
ATC AAG AAA AAT GTC TTA GCG AGG ACT GGT ACT GGC AAC GCA GAG TCG Ile Lys Lys Asn Val Leu Ala Arg Thr Gly Thr Gly Asn Ala Glu Ser				1008	
5	325	330	335		
ATG TCG TGG AAC TGG AGA ACA GAT AGA GAC TTG CTT GAA AAT GGT GCT Met Ser Trp Asn Trp Arg Thr Asp Arg Asp Leu Leu Glu Asn Gly Ala				1056	
	340	345	350		
10	ATT TTT CTC CCA TCC GGG TCT GAT CCA GTG CTA ACC CCT GAG CAA AAA Ile Phe Leu Pro Ser Gly Ser Asp Pro Val Leu Thr Pro Glu Gln Lys				1104
	355	360	365		
15	GCA GGG ATG ATT CCA GCT GAA CCA GGA GAA GCC GTT CTA AGA CTC ACT Ala Gly Met Ile Pro Ala Glu Pro Gly Glu Ala Val Leu Arg Leu Thr				1152
	370	375	380		
20	AGT AGT GCT GGT GTA CTC TCA TGC CAT CAA GGA GCA CCT TGC TAA Ser Ser Ala Gly Val Leu Ser Cys His Gln Gly Ala Pro Cys				1197
	385	390	395		
GCACCTGGCC AATTCTAAAG CTTTTATAAT AATCATAAAAT ACTTATTTA TTTTATTTT					1260
25	GATATTTAT ATGAACCATT ACGTTCAAGT ACTCTATTAA CATGTTTAA ATTCTATAAGA				1320
	GTTTATTGAT AAAAAAAA AAAACCGAAT TC				1349

30 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 398 amino acids
- (B) TYPE: amino acid
- (C) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

40	Met Gly Ile Lys His Cys Cys Tyr Ile Leu Tyr Phe Thr Leu Ala Leu			
	1	5	10	15
45	Val Thr Leu Leu Gln Pro Val Arg Ser Ala Glu Asp Val Glu Glu Phe			
	20	25	30	
50	Leu Pro Ser Ala Asn Glu Thr Arg Arg Ser Leu Lys Ala Cys Glu Ala			
	35	40	45	
55	Asn Arg Gln Ala Leu Ala Asp Cys Ala Gln Gly Phe Ala Lys Gly Thr			
	65	70	75	80
60	Tyr Gly Gly Lys His Gly Asp Val Tyr Thr Val Thr Ser Asp Lys Asp			
	85	90	95	
Asp Asp Val Ala Asn Pro Lys Glu Gly Thr Leu Arg Phe Ala Ala Ala				

- 61 -

	100	105	110
Gln Asn Arg Pro Leu Trp Ile Ile Phe Lys Arg Asn Met Val Ile His			
	115	120	125
5	Leu Asn Gln Glu Leu Val Val Asn Ser Asp Lys Thr Ile Asp Gly Arg		
	130	135	140
Gly Val Lys Val Asn Ile Val Asn Ala Gly Leu Thr Leu Met Asn Val			
10	145	150	155
	160		
Lys Asn Ile Ile His Asn Ile Asn His Asp Ile Lys Val Cys			
	165	170	175
15	Pro Gly Gly Met Ile Lys Ser Asn Asp Gly Pro Pro Ile Leu Arg Gln		
	180	185	190
Gln Ser Asp Gly Asp Ala Ile Asn Val Ala Gly Ser Ser Gln Ile Trp			
	195	200	205
20	Ile Asp His Cys Ser Leu Ser Lys Ala Ser Asp Gly Leu Leu Asp Ile		
	210	215	220
Thr Leu Gly Ser Ser His Val Thr Val Ser Asn Cys Lys Phe Thr Gln			
25	225	230	235
	240		
His Gln Phe Val Leu Leu Leu Gly Ala Asp Asp Thr His Tyr Gln Asp			
	245	250	255
30	Lys Gly Met Leu Ala Thr Val Ala Phe Asn Met Phe Thr Asp His Val		
	260	265	270
Asp Gln Arg Met Pro Arg Cys Arg Phe Gly Phe Phe Gln Val Val Asn			
	275	280	285
35	Asn Asn Tyr Asp Arg Trp Gly Thr Tyr Ala Ile Gly Gly Ser Ser Ala		
	290	295	300
Pro Thr Ile Leu Ser Gln Gly Asn Arg Phe Phe Ala Pro Asp Asp Ile			
40	305	310	315
	320		
Ile Lys Lys Asn Val Leu Ala Arg Thr Gly Thr Gly Asn Ala Glu Ser			
	325	330	335
45	Met Ser Trp Asn Trp Arg Thr Asp Arg Asp Leu Leu Glu Asn Gly Ala		
	340	345	350
Ile Phe Leu Pro Ser Gly Ser Asp Pro Val Leu Thr Pro Glu Gln Lys			
	355	360	365
50	Ala Gly Met Ile Pro Ala Glu Pro Gly Glu Ala Val Leu Arg Leu Thr		
	370	375	380
Ser Ser Ala Gly Val Leu Ser Cys His Gln Gly Ala Pro Cys			
55	385	390	395

(2) INFORMATION FOR SEQ ID NO:5:

- 62 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1320 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA

10 (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1191

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATG	GGG	ATC	AAA	CAA	TGT	TGT	TAC	ATC	TTG	TAT	TTT	ACC	TTA	GCA	CTT	48	
Met	Gly	Ile	Lys	Gln	Cys	Cys	Tyr	Ile	Leu	Tyr	Phe	Thr	Leu	Ala	Leu		
1					5				10				15				
20	GTC	GCT	TTG	CTG	CAA	CCT	GTT	CGT	TCT	GCC	GAA	GGT	GTC	GGG	GAA	ATC	96
Val	Ala	Leu	Leu	Gln	Pro	Val	Arg	Ser	Ala	Glu	Gly	Val	Gly	Glu	Ile		
					20			25				30					
25	TTA	CCT	TCA	GTT	AAC	GAA	ACG	AGG	AGC	CTG	CAA	GCA	TGT	GAA	GCA	CTC	144
Leu	Pro	Ser	Val	Asn	Glu	Thr	Arg	Ser	Leu	Gln	Ala	Cys	Glu	Ala	Leu		
					35			40			45						
30	AAC	ATT	ATA	GAC	AAG	TGC	TGG	AGG	GGC	AAA	GCC	GAT	TGG	GAG	AAC	AAC	192
Asn	Ile	Ile	Asp	Lys	Cys	Trp	Arg	Gly	Lys	Ala	Asp	Trp	Glu	Asn	Asn		
					50			55			60						
35	CGA	CAA	GCG	TTA	GCC	GAC	TGT	GCC	CAA	GGT	TTT	GCA	AAG	GGA	ACC	TAC	240
Arg	Gln	Ala	Leu	Ala	Asp	Cys	Ala	Gln	Gly	Phe	Ala	Lys	Gly	Thr	Tyr		
					65			70			75			80			
40	GGC	GGA	AAA	TGG	GGT	GAT	GTC	TAC	ACG	GTC	ACC	AGC	AAT	CTA	GAT	GAT	288
Gly	Gly	Lys	Trp	Gly	Asp	Val	Tyr	Thr	Val	Thr	Ser	Asn	Leu	Asp	Asp		
					85			90			95						
45	GAT	GTT	GCA	AAT	CCA	AAA	GAA	GGC	ACA	CTC	CGG	TTT	GCT	GCC	GCC	CAA	336
Asp	Val	Ala	Asn	Pro	Lys	Glu	Gly	Thr	Leu	Arg	Phe	Ala	Ala	Gln			
					100			105			110						
50	AAC	AGG	CCC	TTG	TGG	ATC	ATT	TTT	AAA	AAT	GAT	ATG	GTG	ATT	AAT	TTG	384
Asn	Arg	Pro	Leu	Trp	Ile	Ile	Phe	Lys	Asn	Asp	Met	Val	Ile	Asn	Leu		
					115			120			125						
55	AAT	CAA	GAG	CTT	GTC	GTA	AAC	AGC	GAC	AAG	ACC	ATC	GAT	GGC	CGA	GGG	432
Asn	Gln	Glu	Leu	Val	Val	Asn	Ser	Asp	Lys	Thr	Ile	Asp	Gly	Arg	Gly		
					130			135			140						
60	GTG	AAA	GTT	GAA	ATC	ATT	AAC	GGA	GGT	CTC	ACC	CTC	ATG	AAT	GTC	AAG	480
Val	Lys	Val	Glu	Ile	Ile	Asn	Gly	Gly	Leu	Thr	Leu	Met	Asn	Val	Lys		
									150		155		160				
65	AAT	ATA	ATC	ATT	CAT	AAC	ATA	AAT	ATC	CAT	GAT	GTT	AAA	GTG	CTT	CCA	528
Asn	Ile	Ile	Ile	His	Asn	Ile	Asn	Ile	Asp	Val	Lys	Val	Leu	Pro			
					165			170			175						

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5	GGA GGC ATG ATT AAG TCC AAC GAT GGT CCA CCA ATT TTA AGA CAA GCA Gly Gly Met Ile Lys Ser Asn Asp Gly Pro Pro Ile Leu Arg Gln Ala 180 185 190	576
10	AGT GAT GGG GAT ACT ATA AAT GTT GCT GGT AGT TCC CAA ATA TGG ATA Ser Asp Gly Asp Thr Ile Asn Val Ala Gly Ser Ser Gln Ile Trp Ile 195 200 205	624
15	GAC CAT TGC TCA CTC AGC AAG TCT TTC GAT GGG CTG GTC GAT GTC ACC Asp His Cys Ser Leu Ser Lys Ser Phe Asp Gly Leu Val Asp Val Thr 210 215 220	672
20	CTC GGT AGC ACA CAC GTG ACC ATT TCC AAC TGC AAA TTC ACC CAA CAG Leu Gly Ser Thr His Val Thr Ile Ser Asn Cys Lys Phe Thr Gln Gln 225 230 235 240	720
25	TCA AAA GCA ATA TTG TTG GGA GCA GAT GAC ACC CAT GTT CAA GAT AAA Ser Lys Ala Ile Leu Leu Gly Ala Asp Asp Thr His Val Gln Asp Lys 245 250 255	768
30	GGA ATG CTA GCA ACG GTC GCT TTC AAC ATG TTC ACC GAT AAC GTT GAC Gly Met Leu Ala Thr Val Ala Phe Asn Met Phe Thr Asp Asn Val Asp 260 265 270	816
35	CAA AGA ATG CCT AGA TGT CGA TTT GGG TTT TTC CAA GTT GTT AAC AAC Gln Arg Met Pro Arg Cys Arg Phe Gly Phe Phe Gln Val Val Asn Asn 275 280 285	864
40	AAC TAC GAC AGA TGG GGA ACG TAC GCC ATA GGT GGT AGC TCG GCC CCA Asn Tyr Asp Arg Trp Gly Thr Tyr Ala Ile Gly Ser Ser Ala Pro 290 295 300	912
45	ACT ATA CTC TGC CAA GGG AAC AGA TTC TTG GCC CCT GAT GAT CAG ATC Thr Ile Leu Cys Gln Gly Asn Arg Phe Leu Ala Pro Asp Asp Gln Ile 305 310 315 320	960
50	AAG AAA AAT GTC CTA GCG AGG ACT GGT ACA GGC GCT GCT GAG TCG ATG Lys Lys Asn Val Leu Ala Arg Thr Gly Thr Gly Ala Ala Glu Ser Met 325 330 335	1008
55	GCG TGG AAC TGG AGA TCT GAT AAA GAC TTG CTT GAA AAT GGT GCT ATT Ala Trp Asn Trp Arg Ser Asp Lys Asp Leu Leu Glu Asn Gly Ala Ile 340 345 350	1056
60	TTT GTT ACA TCT GGG TCT GAT CCA GTG CTA ACC CCT GTT CAA AGC GCA Phe Val Thr Ser Gly Ser Asp Pro Val Leu Thr Pro Val Gln Ser Ala 355 360 365	1104
65	GGG ATG ATT CCA GCT GAA CCA GGA GAA GCC GCT ATA AAA CTC ACT AGT Gly Met Ile Pro Ala Glu Pro Gly Glu Ala Ala Ile Lys Leu Thr Ser 370 375 380	1152
70	AGT GCT GGT GTA TTC TCA TGC CGT CCT GGA GCA CCT TGC TAAGCACCCT Ser Ala Gly Val Phe Ser Cys Arg Pro Gly Ala Pro Cys 385 390 395	1201
75	GCCAATTCTC CTAAGCTTTT GCAATGATCA AAAATACTTT TTTATTTAT TTTTAATATT	1261

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TTATATGTAC TGGAAATGAA CCATTACCTT CTAGTACTCT ATAACATGTT TTGCATTTA

1320

## (2) INFORMATION FOR SEQ ID NO:6:

5

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 397 amino acids
- (B) TYPE: amino acid
- (C) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

15 Met Gly Ile Lys Gln Cys Cys Tyr Ile Leu Tyr Phe Thr Leu Ala Leu  
 1 5 10 15

Val Ala Leu Leu Gln Pro Val Arg Ser Ala Glu Gly Val Gly Glu Ile  
 20 25 30

20 Leu Pro Ser Val Asn Glu Thr Arg Ser Leu Gln Ala Cys Glu Ala Leu  
 35 40 45

25 Asn Ile Ile Asp Lys Cys Trp Arg Gly Lys Ala Asp Trp Glu Asn Asn  
 50 55 60

Arg Gln Ala Leu Ala Asp Cys Ala Gln Gly Phe Ala Lys Gly Thr Tyr  
 65 70 75 80

30 Gly Gly Lys Trp Gly Asp Val Tyr Thr Val Thr Ser Asn Leu Asp Asp  
 85 90 95

Asp Val Ala Asn Pro Lys Glu Gly Thr Leu Arg Phe Ala Ala Ala Gln  
 100 105 110

35 Asn Arg Pro Leu Trp Ile Ile Phe Lys Asn Asp Met Val Ile Asn Leu  
 115 120 125

Asn Gln Glu Leu Val Val Asn Ser Asp Lys Thr Ile Asp Gly Arg Gly  
 130 135 140

40 Val Lys Val Glu Ile Ile Asn Gly Gly Leu Thr Leu Met Asn Val Lys  
 145 150 155 160

45 Asn Ile Ile Ile His Asn Ile Asn His Asp Val Lys Val Leu Pro  
 165 170 175

Gly Gly Met Ile Lys Ser Asn Asp Gly Pro Pro Ile Leu Arg Gln Ala  
 180 185 190

50 Ser Asp Gly Asp Thr Ile Asn Val Ala Gly Ser Ser Gln Ile Trp Ile  
 195 200 205

Asp His Cys Ser Leu Ser Lys Ser Phe Asp Gly Leu Val Asp Val Thr  
 210 215 220

55 Leu Gly Ser Thr His Val Thr Ile Ser Asn Cys Lys Phe Thr Gln Gln  
 225 230 235 240

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Ser Lys Ala Ile Leu Leu Gly Ala Asp Asp Thr His Val Gln Asp Lys  
 245 250 255

5 Gly Met Leu Ala Thr Val Ala Phe Asn Met Phe Thr Asp Asn Val Asp  
 260 265 270

Gln Arg Met Pro Arg Cys Arg Phe Gly Phe Phe Gln Val Val Asn Asn  
 275 280 285

10 Asn Tyr Asp Arg Trp Gly Thr Tyr Ala Ile Gly Gly Ser Ser Ala Pro  
 290 295 300

15 Thr Ile Leu Cys Gln Gly Asn Arg Phe Leu Ala Pro Asp Asp Gln Ile  
 305 310 315 320

Lys Lys Asn Val Leu Ala Arg Thr Gly Thr Gly Ala Ala Glu Ser Met  
 325 330 335

20 Ala Trp Asn Trp Arg Ser Asp Lys Asp Leu Leu Glu Asn Gly Ala Ile  
 340 345 350

Phe Val Thr Ser Gly Ser Asp Pro Val Leu Thr Pro Val Gln Ser Ala  
 355 360 365

25 Gly Met Ile Pro Ala Glu Pro Gly Glu Ala Ala Ile Lys Leu Thr Ser  
 370 375 380

30 Ser Ala Gly Val Phe Ser Cys Arg Pro Gly Ala Pro Cys  
 385 390 395

(2) INFORMATION FOR SEQ ID NO:7:

THE SEQUENCE IS A PROTEIN

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 1187 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

45 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..1176

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

50 ATG GGG ATC AAA CAC TGT TGT TAC ATC TTG TAT TTT ACC TTA GCC CTT  
 Met Gly Ile Lys His Cys Cys Tyr Ile Leu Tyr Phe Thr Leu Ala Leu  
 1 5 10 15

55 GTC ACT TTG CTG CAA CCT GTT CGT TCT GCC GAA GAT CTC CAG GAA ATC  
 Val Thr Leu Leu Gln Pro Val Arg Ser Ala Glu Asp Leu Gln Glu Ile  
 20 25 30

TTA CCT TCA GCT AAC GAA ACA AGG AGC CTG ACA ACA TGT GGA ACA TAC  
 Leu Pro Ser Ala Asn Glu Thr Arg Ser Leu Thr Thr Cys Gly Thr Tyr  
 144

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	35	40	45	
				192
	AAC ATT ATA GAC GGG TGC TGG AGG GGC AAA GCC GAT TGG GCG GAA AAC			
	Asn Ile Ile Asp Gly Cys Trp Arg Gly Lys Ala Asp Trp Ala Glu Asn			
5	50	55	60	
				240
	CGA AAA GCG TTA GCC GAT TGT GCC CAA GGT TTT GCA AAG GGA ACA ATC			
	Arg Lys Ala Leu Ala Asp Cys Ala Gln Gly Phe Ala Lys Gly Thr Ile			
	65	70	75	80
10				288
	GGC GGA AAA GAT GGT GAT ATA TAC ACG GTC ACC AGT GAG CTA GAT GAT			
	Gly Gly Lys Asp Gly Asp Ile Tyr Thr Val Thr Ser Glu Leu Asp Asp			
	85	90		95
15				336
	GAT GTT GCA AAT CCA AAA GAA GGC ACA CTC CGG TTT GGT GCC GCC CAA			
	Asp Val Ala Asn Pro Lys Glu Gly Thr Leu Arg Phe Gly Ala Ala Gln			
	100	105		110
				384
20				
	AAC AGG CCC TTG TGG ATT ATT TTT GAA AGA GAT ATG GTG ATT CGT TTG			
	Asn Arg Pro Leu Trp Ile Ile Phe Glu Arg Asp Met Val Ile Arg Leu			
	115	120		125
				432
25				
	GAT AGA GAG TTG GCT ATA AAC AAC GAC AAG ACC ATC GAT GGC CGA GGG			
	Asp Arg Glu Leu Ala Ile Asn Asn Asp Lys Thr Ile Asp Gly Arg Gly			
	130	135		140
				480
30				
	GCG AAA GTT GAA ATC ATT AAC GCT GGT TTC GCC ATC TAT AAT GTC AAG			
	Ala Lys Val Glu Ile Ile Asn Ala Gly Phe Ala Ile Tyr Asn Val Lys			
	145	150		160
				528
35				
	AAT ATA ATC ATT CAT AAC ATA ATT ATG CAT GAT ATT GTA GTG AAT CCA			
	Asn Ile Ile Ile His Asn Ile Ile Met His Asp Ile Val Val Asn Pro			
	165	170		175
				576
40				
	GGA GGC CTG ATT AAG TCC CAC GAT GGT CCA CCA GTT CCA AGA AAG GGT			
	Gly Gly Leu Ile Lys Ser His Asp Gly Pro Pro Val Pro Arg Lys Gly			
	180	185		190
				624
45				
	AGT GAT GGT GAT GCT ATA GGT ATT TCT GGT TCA CAA ATA TGG ATC			
	Ser Asp Gly Asp Ala Ile Gly Ile Ser Gly Gly Ser Gln Ile Trp Ile			
	195	200		205
				672
50				
	GAC CAT TGC TCC CTC AGT AAG GCT GTT GAT GGG CTA ATC GAT GCT AAA			
	Asp His Cys Ser Leu Ser Lys Ala Val Asp Gly Leu Ile Asp Ala Lys			
	210	215		220
				720
55				
	CAC GGC AGC ACA CAC TTC ACC GTT TCT AAC TGC TTA TTC ACC CAA CAC			
	His Gly Ser Thr His Phe Thr Val Ser Asn Cys Leu Phe Thr Gln His			
	225	230		240
				768
60				
	CAA TAT TTA TTA TTG TTC TGG GAT TTT GAC GAG CGA GGC ATG CTA TGT			
	Gln Tyr Leu Leu Leu Phe Trp Asp Phe Asp Glu Arg Gly Met Leu Cys			
	245	250		255
				816
65				
	ACG GTC GCA TTC AAC AAG TTC ACT GAT AAC GTT GAC CAA AGA ATG CCT			
	Thr Val Ala Phe Asn Lys Phe Thr Asp Asn Val Asp Gln Arg Met Pro			
	260	265		270
				864
	AAC TTA CGA CAT GGG TTT GTC CAA GTC GTT AAC AAC AAC TAC GAA AGA			

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	Asn Leu Arg His Gly Phe Val Gln Val Val Asn Asn Asn Tyr Glu Arg			
	275	280	285	
5	TGG GGA TCG TAC GCC CTC GGT GGT AGC GCA GGC CCA ACC ATA CTT AGC			912
	Trp Gly Ser Tyr Ala Leu Gly Gly Ser Ala Gly Pro Thr Ile Leu Ser			
	290	295	300	
10	CAA GGG AAC AGA TTC TTA GCC TCC GAT ATC AAG AAA GAG GTC GTA GGG			960
	Gln Gly Asn Arg Phe Leu Ala Ser Asp Ile Lys Lys Glu Val Val Gly			
	305	310	315	320
15	AGG TAT GGT GAA TCC GCC ATG TCA GAG TCG ATT AAT TGG AAC TGG AGA			1008
	Arg Tyr Gly Glu Ser Ala Met Ser Glu Ser Ile Asn Trp Asn Trp Arg			
	325	330	335	
20	TCG TAT ATG GAC GTA TTT GAA AAT GGT GCT ATT TTT GTT CCA TCC GGG			1056
	Ser Tyr Met Asp Val Phe Glu Asn Gly Ala Ile Phe Val Pro Ser Gly			
	340	345	350	
25	GTT GAT CCA GTG CTA ACC CCT GAG CAA AAC GCA GGG ATG ATT CCA GCC			1104
	Val Asp Pro Val Leu Thr Pro Glu Gln Asn Ala Gly Met Ile Pro Ala			
	355	360	365	
30	GAA CCA GGA GAA GCC GTT CTA AGA CTC ACT AGT AGT GCT GGT GTC CTC			1152
	Glu Pro Gly Glu Ala Val Leu Arg Leu Thr Ser Ser Ala Gly Val Leu			
	370	375	380	
	TCA TGC CAA CCT GGA GCA CCT TGC TAAGCACTGC A			1187
	Ser Cys Gln Pro Gly Ala Pro Cys			
	385	390		

## (2) INFORMATION FOR SEQ ID NO:8:

35	(i) SEQUENCE CHARACTERISTICS:															
	(A) LENGTH: 392 amino acids															
	(B) TYPE: amino acid															
	(D) TOPOLOGY: linear															
40	(ii) MOLECULE TYPE: protein															
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:															
45	Met	Gly	Ile	Lys His Cys Cys Tyr Ile Leu Tyr Phe Thr Leu Ala Leu												
	1		5		10	15										
	Val	Thr	Leu	Leu	Gln	Pro	Val	Arg	Ser	Ala	Glu	Asp	Leu	Gln	Glu	Ile
			20		25		30									
50	Leu	Pro	Ser	Ala	Asn	Glu	Thr	Arg	Ser	Leu	Thr	Thr	Cys	Gly	Thr	Tyr
	35		40		45											
55	Asn	Ile	Ile	Asp	Gly	Cys	Trp	Arg	Gly	Lys	Ala	Asp	Trp	Ala	Glu	Asn
	50		55		60											
	Arg	Lys	Ala	Leu	Ala	Asp	Cys	Ala	Gln	Gly	Phe	Ala	Lys	Gly	Thr	Ile
	65		70		75								80			
	Gly	Gly	Lys	Asp	Gly	Asp	Ile	Tyr	Thr	Val	Thr	Ser	Glu	Leu	Asp	Asp

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	85	90	95
Asp Val Ala Asn Pro Lys Glu Gly Thr Leu Arg Phe Gly Ala Ala Gln			
	100	105	110
5	Asn Arg Pro Leu Trp Ile Ile Phe Glu Arg Asp Met Val Ile Arg Leu		
	115	120	125
10	Asp Arg Glu Leu Ala Ile Asn Asn Asp Lys Thr Ile Asp Gly Arg Gly		
	130	135	140
Ala Lys Val Glu Ile Ile Asn Ala Gly Phe Ala Ile Tyr Asn Val Lys			
	145	150	155
15	160		
	Asn Ile Ile Ile His Asn Ile Ile Met His Asp Ile Val Val Asn Pro		
	165	170	175
Gly Gly Leu Ile Lys Ser His Asp Gly Pro Pro Val Pro Arg Lys Gly			
	180	185	190
20	Ser Asp Gly Asp Ala Ile Gly Ile Ser Gly Gly Ser Gln Ile Trp Ile		
	195	200	205
Asp His Cys Ser Leu Ser Lys Ala Val Asp Gly Leu Ile Asp Ala Lys			
25	210	215	220
His Gly Ser Thr His Phe Thr Val Ser Asn Cys Leu Phe Thr Gln His			
	225	230	235
30	240		
	Gln Tyr Leu Leu Leu Phe Trp Asp Phe Asp Glu Arg Gly Met Leu Cys		
	245	250	255
Thr Val Ala Phe Asn Lys Phe Thr Asp Asn Val Asp Gln Arg Met Pro			
	260	265	270
35	275		
	Asn Leu Arg His Gly Phe Val Gln Val Val Asn Asn Tyr Glu Arg		
	275	280	285
Trp Gly Ser Tyr Ala Leu Gly Gly Ser Ala Gly Pro Thr Ile Leu Ser			
40	290	295	300
Gln Gly Asn Arg Phe Leu Ala Ser Asp Ile Lys Lys Glu Val Val Gly			
	305	310	315
45	320		
	Gly Asn Arg Phe Leu Ala Ser Asp Ile Lys Lys Glu Val Val Gly		
	325	330	335
Ser Tyr Met Asp Val Phe Glu Asn Gly Ala Ile Phe Val Pro Ser Gly			
	340	345	350
50	355		
	Val Asp Pro Val Leu Thr Pro Glu Gln Asn Ala Gly Met Ile Pro Ala		
	355	360	365
Glu Pro Gly Glu Ala Val Leu Arg Leu Thr Ser Ser Ala Gly Val Leu			
55	370	375	380
Ser Cys Gln Pro Gly Ala Pro Cys			
	385	390	

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## (2) INFORMATION FOR SEQ ID NO:9:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1395 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

15 (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1191

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

20	ATG GGG ATC AAA CAT TGT TGT TAC ATC TTG TAT TTT ACC TTA GCA CTT Met Gly Ile Lys His Cys Cys Tyr Ile Leu Tyr Phe Thr Leu Ala Leu 1 5 10 15	48
25	GTC ACT TTG GTG CAA GCT GGA CGT CTT GGC GAA GAG GTC GAC ATC TTA Val Thr Leu Val Gln Ala Gly Arg Leu Gly Glu Glu Val Asp Ile Leu 20 25 30	96
30	CCT TCA CCT AAC GAT ACA AGG AGG AGC CTG CAA GGA TGT GAA GCA CAC Pro Ser Pro Asn Asp Thr Arg Arg Ser Leu Gln Gly Cys Glu Ala His 35 40 45	144
35	AAC ATT ATA GAC AAG TGT TGG AGG TGC AAA CCC GAT TGG GCG GAG AAC Asn Ile Ile Asp Lys Cys Trp Arg Cys Lys Pro Asp Trp Ala Glu Asn 50 55 60	192
40	CGA CAA GCG TTA GGC GAT TGT GCG CAA GGT TTT GGA AAG GCA ACT CAC Arg Gln Ala Leu Gly Asp Cys Ala Gln Gly Phe Gly Lys Ala Thr His 65 70 75 80	240
45	GGC GGA AAA TGG GGT GAT ATC TAC ATG GTC ACA AGT GAT CAG GAT GAT Gly Gly Lys Trp Gly Asp Ile Tyr Met Val Thr Ser Asp Gln Asp Asp 85 90 95	288
50	GAT GTT GTA AAT CCA AAA GAA GGC ACA CTC CGG TTC GGT GCT ACC CAG Asp Val Val Asn Pro Lys Glu Gly Thr Leu Arg Phe Gly Ala Thr Gln 100 105 110	336
55	GAC AGG CCC TTG TGG ATC ATT TTT CAA AGA GAT ATG ATT ATT TAT TTG Asp Arg Pro Leu Trp Ile Ile Phe Gln Arg Asp Met Ile Ile Tyr Leu 115 120 125	384
60	CAA CAA GAG ATG GTC GTA ACC AGC GAC ACC ACC ATT GAT GGT CGA GGG Gln Gln Glu Met Val Val Thr Ser Asp Thr Thr Ile Asp Gly Arg Gly 130 135 140	432
65	GCG AAA GTT GAG CTC GTT TAT GGA GGT ATC ACC CTC ATG AAT GTC AAG Ala Lys Val Glu Leu Val Tyr Gly Gly Ile Thr Leu Met Asn Val Lys 145 150 155 160	480

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AAT GTA ATC ATT CAC AAC ATA GAT ATC CAT GAT GTT AGA GTG CTT CCA Asn Val Ile Ile His Asn Ile Asp Ile His Asp Val Arg Val Leu Pro 165 170 175	528
5 GGA GGT AGG ATT AAG TCC AAT GGT GGT CCA GCC ATA CCA AGA CAT CAG Gly Gly Arg Ile Lys Ser Asn Gly Gly Pro Ala Ile Pro Arg His Gln 180 185 190	576
10 AGT GAT GGT GAT GCT ATC CAT GTT ACG GGT AGT TCA GAC ATA TGG ATC Ser Asp Gly Asp Ala Ile His Val Thr Gly Ser Ser Asp Ile Trp Ile 195 200 205	624
15 GAC CAT TGC ACG CTC AGT AAG TCA TTT GAT GGG CTC GTC GAT GTC AAC Asp His Cys Thr Leu Ser Lys Ser Phe Asp Gly Leu Val Asp Val Asn 210 215 220	672
20 TGG GGC AGC ACA GGA GTA ACC ATT TCC AAC TGC AAA TTC ACC CAC CAC Trp Gly Ser Thr Gly Val Thr Ile Ser Asn Cys Lys Phe Thr His His 225 230 235 240	720
25 GAA AAA GCT GTT TTG CTC GGG GCT AGT GAC ACG CAT TTT CAA GAT CTG Glu Lys Ala Val Leu Leu Gly Ala Ser Asp Thr His Phe Gln Asp Leu 245 250 255	768
30 25 GAA ATG CAT GTA ACG CTT GCA TAC AAC ATC TTC ACC AAT ACC GTT CAC Lys Met His Val Thr Leu Ala Tyr Asn Ile Phe Thr Asn Thr Val His 260 265 270	816
35 27 GAA AGA ATG CCC AGA TGC CGA TTT GGG TTT TTC CAA ATC GTT AAC AAC Glu Arg Met Pro Arg Cys Arg Phe Gly Phe Phe Gln Ile Val Asn Asn 275 280 285	864
40 28 TTC TAC GAC AGA TGG GAT AAG TAC GCC ATC GGT GGT AGC TCG AAC CCT Phe Tyr Asp Arg Trp Asp Lys Tyr Ala Ile Gly Ser Ser Asn Pro 290 295 300	912
45 29 ACT ATT CTC AGC CAA GGG AAC AAA TTC GTG GCC CCC GAT TTC ATT TAC Thr Ile Leu Ser Gln Gly Asn Lys Phe Val Ala Pro Asp Phe Ile Tyr 305 310 315 320	960
50 30 AAG AAA AAC GTC TGT CTA AGG ACT GGT GCA CAG GAG CCA GAA TGG ATG Lys Lys Asn Val Cys Leu Arg Thr Gly Ala Gln Glu Pro Glu Trp Met 325 330 335	1008
55 31 ACT TGG AAC TGG AGA ACA CAA AAC GAC GTG CTT GAA AAT GGT GCT ATC Thr Trp Asn Trp Arg Thr Gln Asn Asp Val Leu Glu Asn Gly Ala Ile 340 345 350	1056
55 32 TTT GTG GCA TCT GGG TCT GAT CCA GTG CTA ACC GCT GAA CAA AAT GCA Phe Val Ala Ser Gly Ser Asp Pro Val Leu Thr Ala Glu Gln Asn Ala 355 360 365	1104
55 33 GGC ATG ATG CAA GCT GAA CCG GGA GAT ATG GTT CCA CAA CTC ACC ATG Gly Met Met Gln Ala Glu Pro Gly Asp Met Val Pro Gln Leu Thr Met 370 375 380	1152
55 34 AAT GCA GGT GTA CTC ACA TGC TCG CCT GGA GCA CCT TGC TAAGCACCTG Asn Ala Gly Val Leu Thr Cys Ser Pro Gly Ala Pro Cys 385 390 395	1201

	GCCAAATTCCCT ATGCAACGAT CATAAATACT TGCTCACCAT AAGTGTTCAT TTGATTAGAT	1261
5	TTGGACACGA ATGATGTAAC CGATTCGTCT GAATTATGAT TTGTTTGAT TCTCAGTTTC	1321
	ATAATATGGC TTCTTGAGAG CAAAATTAGA GAAGAGTGTC TTTGATCAAC TACATTTAT	1381
	GGTTTTTATA TTAA	1395

10

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

15	(A) LENGTH: 397 amino acids
	(B) TYPE: amino acid
	(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## 20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Gly Ile Lys His Cys Cys Tyr Ile Leu Tyr Phe Thr Leu Ala Leu			
1	5	10	15

25 Val Thr Leu Val Gln Ala Gly Arg Leu Gly Glu Glu Val Asp Ile Leu			
	20	25	30

Pro Ser Pro Asn Asp Thr Arg Arg Ser Leu Gln Gly Cys Glu Ala His		
35	40	45

30 Asn Ile Ile Asp Lys Cys Trp Arg Cys Lys Pro Asp Trp Ala Glu Asn		
50	55	60

35 Arg Gln Ala Leu Gly Asp Cys Ala Gln Gly Phe Gly Lys Ala Thr His			
65	70	75	80

Gly Gly Lys Trp Gly Asp Ile Tyr Met Val Thr Ser Asp Gln Asp Asp		
85	90	95

40 Asp Val Val Asn Pro Lys Glu Gly Thr Leu Arg Phe Gly Ala Thr Gln		
100	105	110

Asp Arg Pro Leu Trp Ile Ile Phe Gln Arg Asp Met Ile Ile Tyr Leu		
115	120	125

45 Gln Gln Glu Met Val Val Thr Ser Asp Thr Thr Ile Asp Gly Arg Gly		
130	135	140

50 Ala Lys Val Glu Leu Val Tyr Gly Ile Thr Leu Met Asn Val Lys			
145	150	155	160

Asn Val Ile Ile His Asn Ile Asp Ile His Asp Val Arg Val Leu Pro		
165	170	175

55 Gly Gly Arg Ile Lys Ser Asn Gly Gly Pro Ala Ile Pro Arg His Gln		
180	185	190

Ser Asp Gly Asp Ala Ile His Val Thr Gly Ser Ser Asp Ile Trp Ile		
195	200	205

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Asp His Cys Thr Leu Ser Lys Ser Phe Asp Gly Leu Val Asp Val Asn  
 210 215 220  
 5 Trp Gly Ser Thr Gly Val Thr Ile Ser Asn Cys Lys Phe Thr His His  
 225 230 235 240  
 Glu Lys Ala Val Leu Leu Gly Ala Ser Asp Thr His Phe Gln Asp Leu  
 245 250 255  
 10 Lys Met His Val Thr Leu Ala Tyr Asn Ile Phe Thr Asn Thr Val His  
 260 265 270  
 15 Glu Arg Met Pro Arg Cys Arg Phe Gly Phe Phe Gln Ile Val Asn Asn  
 275 280 285  
 Phe Tyr Asp Arg Trp Asp Lys Tyr Ala Ile Gly Gly Ser Ser Asn Pro  
 290 295 300  
 20 Thr Ile Leu Ser Gln Gly Asn Lys Phe Val Ala Pro Asp Phe Ile Tyr  
 305 310 315 320  
 Lys Lys Asn Val Cys Leu Arg Thr Gly Ala Gln Glu Pro Glu Trp Met  
 325 330 335  
 25 Thr Trp Asn Trp Arg Thr Gln Asn Asp Val Leu Glu Asn Gly Ala Ile  
 340 345 350  
 Phe Val Ala Ser Gly Ser Asp Pro Val Leu Thr Ala Glu Gln Asn Ala  
 355 360 365  
 30 Gly Met Met Gln Ala Glu Pro Gly Asp Met Val Pro Gln Leu Thr Met  
 370 375 380  
 35 Asn Ala Gly Val Leu Thr Cys Ser Pro Gly Ala Pro Cys  
 385 390 395

## (2) INFORMATION FOR SEQ ID NO:11:

40

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 312 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: cDNA

50

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..276

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATG TCG ATT CTT GGA GGA ATT ACC GAA GTT AAA GAC AAT GAT AAC AGC  
 Met Ser Ile Leu Gly Gly Ile Thr Glu Val Lys Asp Asn Asp Asn Ser  
 1 5 10 15

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5	GTC GAT TTC GAC GAG CTT GCT AAA TTC GCC ATC GCT GAA CAC AAC AAG Val Asp Phe Asp Glu Leu Ala Lys Phe Ala Ile Ala Glu His Asn Lys 20 25 30	96
10	AAG GAG AAT GCT GCT CTG GAG TTT GGA AAA GTA ATA GAA AAA AAG CAG Lys Glu Asn Ala Ala Leu Glu Phe Gly Lys Val Ile Glu Lys Lys Gln 35 40 45	144
15	CAG GCG GTA CAG GGC ACC ATG TAT TAT ATA AAA GTG GAA GCA AAT GAT Gln Ala Val Gln Gly Thr Met Tyr Tyr Ile Lys Val Glu Ala Asn Asp 50 55 60	192
20	GGT GGT GAG AAG AAA ACT TAT GAA GCC AAG GTG TGG GTT AAG CTA TGG Gly Gly Glu Lys Lys Thr Tyr Glu Ala Lys Val Trp Val Lys Leu Trp 65 70 75 80	240
25	GAA AAT TTC AAG GAA TTG CAG GAA CTC AAA CTT GTT TGATGTTGCC Glu Asn Phe Lys Glu Leu Gln Glu Leu Lys Leu Val 85 90	286
	ACCTCACCTTA ACTCCATATG GACGG	312

25 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 92 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

30	Met Ser Ile Leu Gly Gly Ile Thr Glu Val Lys Asp Asn Asp Asn Ser 1 5 10 15
35	Val Asp Phe Asp Glu Leu Ala Lys Phe Ala Ile Ala Glu His Asn Lys 20 25 30
40	Lys Glu Asn Ala Ala Leu Glu Phe Gly Lys Val Ile Glu Lys Lys Gln 35 40 45
45	Gln Ala Val Gln Gly Thr Met Tyr Tyr Ile Lys Val Glu Ala Asn Asp 50 55 60
50	Gly Gly Glu Lys Lys Thr Tyr Glu Ala Lys Val Trp Val Lys Leu Trp 65 70 75 80
	Glu Asn Phe Lys Glu Leu Gln Glu Leu Lys Leu Val 85 90

55 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

10 Tyr Ile Leu Tyr Phe Thr Leu Ala Leu Val Thr Leu Leu Gln Pro Val  
1 5 10 15

Arg Ser Ala Glu Asp Leu Gln Glu Ile Leu Pro  
20 25

15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 28 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

30 Pro Val Arg Ser Ala Glu Asp Leu Gln Glu Ile Leu Pro Val Asn Glu  
1 5 10 15

Thr Arg Arg Leu Thr Thr Ser Gly Ala Tyr Asn Ile  
20 25

35

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 24 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

50 Glu Thr Arg Arg Ser Leu Lys Thr Ser Gly Ala Tyr Asn Ile Ile Asp  
1 5 10 15

Gly Cys Trp Arg Gly Lys Ala Asp  
20

55

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Glu	Thr	Arg	Arg	Leu	Thr	Thr	Ser	Gly	Ala	Tyr	Asn	Ile	Ile	Asp	Gly
1				5					10					15	

15 Cys Trp Arg Gly Lys Ala Asp  
20

(2) INFORMATION FOR SEQ ID NO:17:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala	Tyr	Asn	Ile	Ile	Asp	Gly	Cys	Trp	Arg	Gly	Lys	Ala	Asp	Trp	Ala
1				5					10				15		

35 Glu Asn Arg Lys Ala Leu Ala Asp Cys Ala Gln Gly  
20 25

40 (2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Arg	Gly	Lys	Ala	Asp	Trp	Ala	Glu	Asn	Arg	Lys	Ala	Leu	Ala	Asp	Cys
1				5				10					15		

55 Ala Gln Gly

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## (2) INFORMATION FOR SEQ ID NO:19:

5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

## 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

15 Gly Lys Ala Asp Trp Ala Glu Asn Arg Cys  
1 5 10

## 20 (2) INFORMATION FOR SEQ ID NO:20:

25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

## 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

35 Gly Lys Ala Asp Trp Ala Glu Asn Arg Lys  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:21:

40 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:  
Ala Glu Asn Arg Lys Ala Leu Ala Asp Cys Ala Gln Gly  
1 5 10

## 55 (2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids

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(B) TYPE: amino acid  
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Lys Ala Leu Ala Asp Cys Ala Gln Gly Phe Gly Lys Gly Thr Val Gly  
1 5 10 15

15 Gly

(2) INFORMATION FOR SEQ ID NO:23:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gly Phe Gly Lys Gly Thr Val Gly Gly Lys Asp Gly Asp Ile Tyr Ser  
1 5 10 15

35 Val Thr

(2) INFORMATION FOR SEQ ID NO:24:

40 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gly Phe Gly Lys Gly Thr Val Gly Gly Lys Asp Gly Asp Ile Tyr Thr  
1 5 10 15

55 Val Thr

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## (2) INFORMATION FOR SEQ ID NO:25:

5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

15 Lys Asp Gly Asp Ile Tyr Ser Val Thr Ser Glu Leu Asp Asp Asp Val  
1 5 10 15

Ala

20 (2) INFORMATION FOR SEQ ID NO:26:

25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

35 Lys Asp Gly Asp Ile Tyr Thr Val Thr Ser Glu Leu Asp Asp Asp Val  
1 5 10 15

Ala

40 (2) INFORMATION FOR SEQ ID NO:27:

45 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

55 Ile Tyr Thr Val Thr Ser Glu Leu Asp Asp Asp  
1 5 10

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## (2) INFORMATION FOR SEQ ID NO:28:

5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

10 (v) FRAGMENT TYPE: internal

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ile Tyr Thr Val Thr Ser Glu Leu Asp Asp Asp Val Ala Asn  
1 5 10

## 20 (2) INFORMATION FOR SEQ ID NO:29:

25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide

30 (v) FRAGMENT TYPE: internal

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ile Tyr Thr Val Thr Ser Glu Leu Asp Asp Asp Val Ala Asn Pro Lys  
1 5 10 15

35 Glu

## 40 (2) INFORMATION FOR SEQ ID NO:30:

45 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide

50 (v) FRAGMENT TYPE: internal

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ile Tyr Ser Val Thr Ser Glu Leu Asp Asp Asp Val Ala Asn Pro Lys  
1 5 10 15

55 Glu Gly Thr Leu  
20

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## (2) INFORMATION FOR SEQ ID NO:31:

5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

15 Ile Tyr Thr Val Thr Ser Glu Leu Asp Asp Asp Val Ala Asn Pro Lys  
1 5 10 15

20 Glu Gly Thr Leu  
20

## (2) INFORMATION FOR SEQ ID NO:32:

25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

35 Asn Pro Lys Glu Gly Thr Leu Arg Phe Gly Ala Ala Gln Asn Arg Pro  
1 5 10 15

40 Leu Trp Ile Ile Phe Glu Arg Asp Met Val Ile Arg Leu  
20 25

## (2) INFORMATION FOR SEQ ID NO:33:

45 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

55 Trp Ile Ile Phe Glu Arg Asp Met Val Ile Arg Leu Asp Lys Glu Met  
1 5 10 15

- 81 -

Val Val Asn Ser  
20

5

## (2) INFORMATION FOR SEQ ID NO:34:

## (i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 19 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15 (v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

20 Leu Asp Lys Glu Met Val Val Asn Ser Asp Lys Thr Ile Asp Gly Arg  
1 5 10 15

Gly Ala Lys

25

## (2) INFORMATION FOR SEQ ID NO:35:

## (i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 26 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35 (v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

40 Asp Lys Thr Ile Asp Gly Arg Gly Ala Lys Val Glu Ile Ile Asn Ala  
1 5 10 15

Gly Phe Thr Leu Asn Gly Val Lys Asn Val

45 20 25

## (2) INFORMATION FOR SEQ ID NO:36:

## 50 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Gly Phe Thr Leu Asn Gly Val Lys Asn Val Ile Ile His Asn Ile Asn  
1 5 10 15

5

Met His Asp Val Lys Val Asn Pro Gly Gly Leu  
20 25

10 (2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: peptide

20

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

His Asp Val Lys Val Asn Pro Gly Gly Leu Ile Lys Ser Asn Asp Gly  
1 5 10 15

25

Pro Ala Ala Pro Arg Ala Gly Ser Asp Gly Asp Ala  
20 25

30

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

40

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Asn Pro Gly Gly Leu Ile Lys Ser Asn Asp Gly Pro Ala Ala Pro Arg  
1 5 10 15

Ala Gly Ser Asp Gly Asp Ala  
20

50

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: peptide

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(v) FRAGMENT TYPE: internal

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ile Lys Ser Asn Asp Gly Pro Ala Ala Pro Arg Ala Gly Ser Asp Gly  
1 5 10 15

10 Asp Ala

(2) INFORMATION FOR SEQ ID NO:40:

15

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Gly Pro Ala Ala Pro Arg Ala Gly Ser Asp Gly Asp Ala  
1 5 10

30

(2) INFORMATION FOR SEQ ID NO:41:

35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Ala Pro Arg Ala Gly Ser Asp Gly Asp Ala Ile Ser Ile  
1 5 10

50 (2) INFORMATION FOR SEQ ID NO:42:

55

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

10

(2) INFORMATION FOR SEQ ID NO:43:

15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide  
(iii) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

(3) INFORMATION FOR SEQ ID NO:44:

35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

(2) INFORMATION FOR SEQ ID NO:45:

55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

- 85 -

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

10 Trp Ile Asp His Cys Ser Leu Ser Lys Ser Val Asp Gly Leu Val Asp  
1 5 10 15Ala Lys Leu Gly Thr Thr Arg Leu Thr Val Ser Asn  
20 25

15

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 27 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

30 Leu Gly Thr Thr Arg Leu Thr Val Ser Asn Ser Leu Phe Thr Gln His  
1 5 10 15Gln Phe Val Leu Leu Phe Gly Ala Gly Asp Glu  
20 25

35

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 28 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

45 (v) FRAGMENT TYPE: internal

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Phe Val Leu Leu Phe Gly Ala Gly Asp Glu Asn Ile Glu Asp Arg Gly  
1 5 10 1555 Met Leu Ala Thr Val Ala Phe Asn Thr Phe Thr Asp  
20 25

(2) INFORMATION FOR SEQ ID NO:48:

- 86 -

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Leu Ala Thr Val Ala Phe Asn Thr Phe Thr Asp Asn Val Asp Gln Arg  
1 5 10 15

15

Met Pro

(2) INFORMATION FOR SEQ ID NO:49:

20

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Phe Thr Asp Asn Val Asp Gln Arg Met Pro Arg Cys Arg His Gly Phe  
1 5 10 15

35

Phe Gln Val Val  
20

40 (2) INFORMATION FOR SEQ ID NO:50:

45

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

55

Arg Cys Arg His Gly Phe Phe Gln Val Val Asn Asn Asn Tyr Asp Lys  
1 5 10 15

Trp Gly Ser Tyr Ala Ile Gly Gly Ser Ala Ser Pro  
20 25

## (2) INFORMATION FOR SEQ ID NO:51:

## 5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

## 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

His Gly Phe Phe Gln Val Val Asn Asn Asn Tyr Asp Lys Trp Gly Ser  
1 5 10 15

Tyr Ala Ile Gly Gly Ser Ala Ser Pro  
20 25

## (2) INFORMATION FOR SEQ ID NO:52:

## 25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

## 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Phe Gln Val Val Asn Asn Asn Tyr Asp Lys Trp Gly Ser Tyr Ala Ile  
1 5 10 15

Gly Gly Ser Ala Ser Pro  
20

## (2) INFORMATION FOR SEQ ID NO:53:

## 45 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

## 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Val Asn Asn Asn Tyr Asp Lys Trp Gly Ser Tyr Ala Ile Gly Gly Ser  
1 5 10 15

Ala Ser Pro

5

(2) INFORMATION FOR SEQ ID NO:54:

10

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 12 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: peptide

15

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Gly Ser Tyr Ala Ile Gly Gly Ser Ala Ser Pro Thr  
1 5 10

25

(2) INFORMATION FOR SEQ ID NO:55:

30

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Gly Ser Tyr Ala Ile Gly Gly Ser Ala Ser Pro Thr Ile Leu Ser Gln  
1 5 10 15

45

(2) INFORMATION FOR SEQ ID NO:56:

50

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Gly Ser Tyr Ala Ile Gly Gly Ser Ala Ser Pro Thr Ile Leu Ser Gln  
1 5 10 15

Gly Asn Arg Phe